Bee diversity, pollination, and fruit production in strawberry agroecosystems

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Abstract

The conversion of a natural ecosystem to an agroecosystem characteristically involves a reduction in biodiversity. As agricultural intensification continues, it is vital to understand how biodiversity loss in farmlands affects the ecosystem functions, such as pollination, required to maintain crop production. A diversity of wild bee species inhabit agricultural lands, and high species richness within crop pollinator assemblages has been linked to enhanced crop yields; however, species richness is often confounded with abundance in studies of pollinator communities. In addition, direct comparisons of pollinator performance among individual bee species have been limited by methodological constraints, which make it challenging to predict how yield will be affected by changes in pollinator community composition. The overarching goal of this thesis is to explore the influence of bee species identity and community composition on pollination and production in agroecosystems, and to examine how elements of agricultural landscapes impact bee communities.

I first compared the performance of wild bees and honey bees (*Apis mellifera* L.) as pollinators of strawberry, through the use of a new pollen deposition measurement technique and controlled bee diversity field experiments. I found that honey bees and wild bees deposited the same amount of pollen per visited flower, but wild bee-pollinated strawberries were larger than honey bee-pollinated strawberries. This suggests that wild bees transferred higher quality (outcross) pollen than honey bees. As the distance travelled by pollinators between successive flower visits can affect the quality of pollen transferred among plants, I then examined foraging behaviour as a mechanism driving this effect. I found that wild bees, especially those in the genus *Lasioglossum*, foraged more erratically, provided more cross-pollination, and produced larger fruit than honey bees for certain strawberry varieties and field designs.

Although wild bees show promise as strawberry pollinators, conventional cultivation methods may limit wild bee abundance on strawberry farms. The ground within most commercial strawberry fields is covered with straw, and increasingly with plastic mulch. This reduces habitat for ground-nesting bees within the crop, so most wild pollinators need to enter the crop from beyond field margins. To examine the influence of field-margin type (forest or hedgerow) on bee community composition and pollinator export to strawberry crops, I conducted bee

diversity surveys on 12 farms, from crop edge to crop centre. I found that wild bee species richness and abundance did not differ between field-margin types. Small wild bee abundance declined significantly from the crop margin to crop centre but pollen deposition did not, which suggests that large-bodied and managed bees were providing the majority of pollination. Although my previous work indicates that small wild bees are more effective pollinators of strawberry on a per-visit basis, their limited foraging ranges suggest they may only pollinate marginal areas, given typical field sizes in our region.

My overall findings showed that honey bee pollination results in lower strawberry yields than wild bee pollination. Management efforts aimed at the maintenance or enhancement of wild pollinator populations may be a cost-effective way to increase both crop yield and biodiversity on strawberry farms. However, species richness did not affect pollination for the strawberries studied here, which highlights that the conservation of biodiversity and ecosystem services are not always equivalent.

Résumé

La transformation d’un écosystème naturel vers un agroécosystème implique généralement une perte de la biodiversité. Dans le contexte actuel d’intensification agricole, il est essentiel de comprendre comment cette érosion de la biodiversité affecte les fonctions écosystémiques requises pour maintenir la productivité agricole. Une grande diversité d’espèces d’abeilles indigènes se trouve sur les terres agricoles, et une communauté de pollinisateurs détenant une richesse spécifique élevée a été associé à un rendement agricole plus fort. Cependant, la richesse spécifique est souvent confondue avec l’abondance des individus dans les études réalisées sur les communautés de pollinisateurs. De plus, des contraintes dans les approches méthodologiques utilisées empêchent une comparaison directe de la performance de différentes espèces de pollinisateurs, ce qui limite notre capacité à prédire comment un changement dans la composition d’une communauté de pollinisateurs affectera la productivité d’une culture agricole. L’objectif principal de cette thèse est de comprendre comment l’identité spécifique des abeilles et la composition d’une communauté de pollinisateurs dans un agroécosystème influencent la pollinisation et la production agricoles, et de déterminer de quelle manière l’aménagement des terres agricoles affecte les communautés d’abeilles.

J’ai premièrement comparé la performance en tant que pollinisateur de fraisiers des abeilles indigènes avec celle des abeilles à miel européennes (*Apis mellifera* L.), à l’aide d’une nouvelle technique de mesure du dépôt de pollen ainsi qu’une étude de terrain contrôlée sur la diversité des abeilles. J’ai ainsi découvert que les abeilles à miel et les abeilles indigènes déposent la même quantité de pollen par visite de fleur, mais que les fraises pollinisées par les abeilles indigènes atteignent une taille supérieure que celles pollinisées par les abeilles à miel. Cela suggère que les abeilles indigènes transfèrent du pollen de meilleure qualité (par la pollinisation croisée) que celui déposé par les abeilles à miel. J’ai ensuite étudié le comportement de butinage des pollinisateurs en tant que processus influençant la qualité du pollen transféré entre les plantes, considérant l’incidence qu’a la distance parcourue par les pollinisateurs entre des visites successives de fleurs sur la qualité du pollen. J’ai constaté que les abeilles indigènes, en particulier celles issues du genre *Lasioglossum,* butinent de façon plus erratique, ce qui facilite davantage la pollinisation croisée et ainsi produit des fruits plus larges que ceux produits

par les fleurs visitées par les abeilles à miel, et ce pour certaines variétés de fraisiers et certaines configurations de champs cultivés.

Malgré le fait que les abeilles indigènes semblent être de bons pollinisateurs pour les cultures de fraises, les méthodes d'agriculture conventionnelle couramment utilisées peuvent limiter l'abondance de ces pollinisateurs dans les champs de fraises. En effet, dans la plupart des champs de fraises commerciaux, de la paille ou souvent même du paillis de plastique recouvre le sol. L'habitat pour les abeilles nichant au sol des champs se trouve donc à être réduit, ce qui signifie que la plupart des pollinisateurs indigènes doivent provenir de l'extérieur du champ. J'ai procédé à des inventaires de la diversité d'abeilles en bordure des champs et au centre des champs sur 12 fermes, afin d'examiner l'influence du type de bordure d'un champ (forêt ou bande végétale) sur la composition des communautés d'abeilles et sur l'exportation de pollinisateurs de la bordure vers le champ de fraises. J'ai constaté que la richesse spécifique des abeilles indigènes ainsi que leur abondance ne changeaient pas selon les différents types de bordures des champs. L'abondance des petites abeilles indigènes diminuait de manière significative de la bordure du champ en allant vers le centre, mais pas la quantité de pollen déposé, ce qui suggère que d'autres abeilles ayant une plus grande masse corporelle ainsi que des abeilles à miel fournissent en majorité le service de pollinisation. Malgré que mes travaux précédents révèlent que les petites abeilles indigènes pollinisent plus efficacement les fraisiers par visite de fleurs, la limite de leur zone de butinage suggère qu'ils pollinisent peut-être seulement les zones périphériques, considérant les grandeurs typiques des champs agricoles dans la région.

Globalement, mes conclusions indiquent que les fraises se développent moins suite à la pollinisation par des abeilles à miel que par des abeilles indigènes. Les efforts d'aménagement visant à maintenir ou à améliorer les conditions pour les populations d'abeilles indigènes peuvent s'agir de moyens rentables afin d'augmenter la productivité des fraisiers et la biodiversité dans les champs. Cependant, la richesse spécifique des communautés d'abeilles n'influence pas la pollinisation des cultures de fraises utilisées pour cette recherche. Cela démontre que la conservation de la biodiversité et des services écosystémiques ne sont pas toujours des facteurs équivalents.

Contributions to knowledge

1. I developed a new pollen measurement technique that allows the flower to remain undisturbed in the field, enabling subsequent fruit analyses. This technique circumvents problems that have been associated with pollen measurement in the past, and enables the analysis of pollen deposited by multiple visitors to one flower. This technique provides a means to deepen our understanding of plant-pollinator relationships, and explore the direct influence of flower visitor richness and interspecies interactions on pollination.

2. I tested how the contribution of pollen by each bee in assemblages of flower visitors affects fruit production. This is the first field study to disentangle the effects of pollen quantity from pollen quality on fruit production in strawberry. Species richness within crop pollinator communities has been known to enhance yield, but few studies have experimentally separated the effects of species richness and abundance, as was done here. I also found that strawberries were smaller when honey bees were part of flower visit sequences, which contradicts previous findings of complementarity between wild and managed strawberry pollinators.

3. I tested hypotheses on the relationship between crop design and bee foraging behaviour as a mechanism leading to enhanced wild bee pollination and fruit production. This is the first examination of the interactive influence of crop row design and foraging behaviour. I found that intercropping strawberry varieties can increase cross-pollination, but this effect was specific to varieties with low self-compatibility.

4. This thesis adds to the limited body of work on pollinator export from field edges and their effect on pollination of the focal crop. I used a field experiment to test the effects of different field-edge types on bee species richness and abundance within strawberry crops. Although small wild bees were found to be highly effective pollinators of strawberry, their limited foraging ranges suggest they may only pollinate marginal areas of fields. This work also provides a description of crop pollinator communities on strawberry farms in Eastern Canada, which is fundamental to better detect changes in bee communities on agricultural lands.

Chapter 1: Introduction and Literature review

1.1 Biodiversity and ecosystem functions: the case of crop pollination

1.1.1 Introduction

Ecological research provides the foundation to understand the natural world. As much of Earth now consists of human-modified landscapes, it is imperative we understand how the organisms that persist in those landscapes maintain the ecosystem functions on which we depend, and how our activities may disrupt those functions. Over the last quarter-century, ecological field experiments have been instrumental in studies of biodiversity and ecosystem functioning (BEF) and have highlighted the role of diversity in enhancing certain functions, such as primary productivity and nutrient cycling. However, there are several challenges to conducting BEF research, such as assembling experimental communities of a known, or fixed number of species. This has led most studies to be conducted on terrestrial plant communities, the results of which may not generalize across systems.

In the literature review that follows, I first discuss the literature and the debate surrounding the relationship between biodiversity and ecosystem functioning (BEF) and some of the challenges associated with conducting BEF experiments. Second is a discussion of pollination in agroecosystems, with particular focus on bees as providers of crop pollination services. Third, I describe the literature surrounding the influence of bee diversity on crop pollination, and how the agricultural landscape affects the composition of crop pollinator communities. I conclude with a detailed discussion of strawberry agroecosystems, and the published works on pollination in strawberry.

1.1.2 Biodiversity and ecosystem functioning

Elton (1958) postulated that an increase in species diversity would enhance ecosystem stability through increased resistance to disease and exotic invasions. However, this concept remained relatively dormant for decades as ecological research at the time was largely focused on the causes of species diversity rather than the effects. Over the past 30 years there has been a notable shift in how we view the extraordinary diversity of life on earth. In addition to biodiversity arising simply as a product of abiotic and biotic forces, ecologists came to recognize that biodiversity sustained and even enhanced the processes necessary to maintain stable ecosystems (i.e. ecosystem functions). Simply put, “the existence of life alters the environment and the diversity of life determines the manner in which life alters the environment” (Naeem 2002). The shift in research focus came about in the 1990s over concern that human-induced biodiversity loss had the potential to disrupt many ecosystem functions and the services we obtain from them (Schulze & Mooney 1993; Jones & Lawton 1994). However, the relationship between biodiversity and ecosystem functioning is complex, and it is difficult to determine which, and how many, species maintain essential ecosystem functions across temporal and spatial scales. Further, the influence of species diversity versus community composition on ecosystem functioning is difficult to disentangle, and is a subject of an ongoing debate (Grime 1997; Loreau 2001; Srivastava & Vellend 2005; Wardle 2016).

Field experiments have been instrumental in exploring the link between biodiversity and ecosystem functions. Large experiments in grasslands have shown that species-rich plant communities have increased primary productivity and drought resistance (Tilman & Downing 1994; Tilman et al. 1996). Similar experiments have found that both primary productivity and soil nitrate uptake depend on plant functional group composition and functional diversity respectively (Hooper & Vitousek 1997; Tilman et al. 1997). Large meta-analyses (Cardinale et al. 2006) have also found consistent positive impacts of species richness on function across trophic groups (primary producers, herbivores, detrivores and predators). However, variability in the results of studies across functions (e.g. primary productivity vs. decomposition) and systems (temperate vs. tropical) has led to disagreement about the general role of biodiversity in ecosystem functioning (Clarke et al. 2017).

BEF relationships can also be masked by selection effects, meaning that the likelihood an ecological community contains the most productive species increases with species richness (Huston 1997, Ives et al. 2004). It is also challenging to separate the effects of species or functional-group richness from that of community composition (Huston 1997; Cardinale et al. 2006). This has led many BEF field experiments to be conducted in artificially created communities in which species or functional richness are varied at random (reviewed in Wardle 2016). These designs create further problems, as species loss is not random in nature and often depends on specific species traits (body size, generation time), as well as geographic range and population densities (McKinney 1997). Additionally, most BEF experiments have been done in plant communities; research on the connection between biodiversity and ecosystem functions is limited at higher trophic levels. Most studies in which increasing species richness has been shown to increase ecosystem functioning have been conducted in controlled small-scale experiments. The lack of BEF field research conducted under realistic conditions means there is still insufficient data to fully understand the complex relationships between biodiversity, ecosystem functions and specific ecosystem services (Cardinale et al. 2012).

Advances in research methodology and additional field research are necessary to determine whether the results of BEF experiments hold under real-world conditions, across multiple scales. Studies of individual and collective species' roles in the preservation of ecosystem functions and associated services are increasingly important as biodiversity continues to decline on a global scale, partially due to the expansion of intensive agriculture (Lanz et al. 2018). Furthering our understanding of the role of biodiversity in ecosystem functioning, and resilience to environmental change, is essential to the preservation of human well-being in the Anthropocene.

1.1.3 Crop pollination services

Agricultural ecosystems (agroecosystems) are ideal systems in which to assess the effects of biodiversity loss on ecosystem functions and ecosystem services, as the conversion of a natural ecosystem to an agroecosystem almost always involves a reduction in biodiversity (Vandermeer et al. 1998; Tscharntke et al. 2005). Ecosystem functions are defined as ecological processes that control the fluxes of energy, nutrients and organic matter through an environment, whereas ecosystem services are the benefits humans receive from these processes (Cardinale et al. 2012). With the continual expansion of agricultural land (Song et al. 2018), it is important to understand how a reduction in biodiversity on farmlands affects the ecosystem functions required to maintain crop production and our food supply systems. Crop production depends upon the combination of human inputs, ecosystem functions, and ecosystem services. Ecosystem functions and services can interact in complex ways. For example, control of florivorous pests can increase crop yield by increasing the potential for pollination over the flower's lifetime (Sutter & Albrecht 2016). While assessments of the relationships between multiple ecosystem services are necessary to maximize service provision and sustainability in crop production (Kennedy et al. 2013), detailed examinations of individual service providers are essential prerequisites to understanding interactions across functions and services. Although biodiversity-based approaches to agroecosystem management are increasingly encouraged (Macfadyen et al. 2012; Landis 2016; Martin et al. 2019), disentangling the influence of community composition and species richness on specific agroecosystem functions remains a challenge to fully comprehending the role of biodiversity in crop production.

Pollination is often cited as an example of an essential agroecosystem function, essential to food production (de Groot et al. 2002; Swift et al. 2004; Mace et al. 2012). It has been categorized both as a "regulating" and as a "provisioning" ecosystem service (Balvanera et al. 2005; Kremen et al. 2007; Fisher & Turner 2008; Brittain et al. 2013). Despite disagreement over whether pollination constitutes an ecosystem function or service, there is a general consensus on the value of pollination to the production of many fruit, vegetable, fodder, and seed crops (Klein et al 2006; Eilers et al. 2011). The reproductive success of pollinator-dependent plants can be limited by insufficient or incompatible pollen transfer by their pollinators (Burd 1994; Aizen and Harder 2007) or by a lack of available resources, such as water or soil nutrients (Haig & Westoby 1988; Burd 2008). With no shortage of resources in agricultural environments, there is reason to believe that plants in agroecosystems may be more prone to pollen-limitation of fruit or seed set (Garibaldi et al. 2011).

Bees are the main flower visitors of many pollinator-dependent crops (Klein et al. 2006). Although the quantity of pollen deposited within a flower must be sufficient to fertilize all its ovules, the quality of the pollen also plays an important role. Low-quality pollen is that which originates from the same source plant (self-pollen) or is not conspecific or viable (Wilcock & Neiland 2002; Aizen & Harder 2007). Self-pollination prohibits reproduction in self-incompatible plants but can also reduce the quality of offspring relative to out-crossed progeny in self-compatible plants (Darwin 1876; Waser & Price; 1991; Barrett & Harder 1996). Among other factors, the quality of the pollen deposited within a flower depends on the mating system of the plant and the foraging behaviour of flower visitors. The temporal or spatial separation of reproductive organs or functions can reduce the frequency of self-pollination, but the movement of pollinators between and within plants is an important determinant of the quality of pollen transferred between flowers (Levin et al. 1971; Levin 1979; Schmitt 1980). However, the influence of bee foraging behaviour on the quality of pollen transferred has been understudied in agroecosystems (but see Raw 2000; Greenleaf & Kremen 2006; Brittain et al. 2013).

The introduction of managed pollinators, such as the European honey bee (*Apis mellifera* L.), has enabled a degree of control over the level of pollination in crop plants. The yields of commercial crops that are highly dependent on animal pollination (e.g., almonds) would not be sustainable at present levels without managed pollinator services. However, fruit and seed production is often found to be positively related to species richness of flower visitors (Klein et al. 2003; Brittain et al. 2013; Garibaldi et al. 2013), though the connection between species richness and pollination is complex. The quantity of pollen deposited by each bee species to a flower is highly variable, and a diversity of functional traits and interactions among bees and flowering plants can influence the amount and type of pollen deposited (Chagnon et al. 1993; Wolfe & Barrett 1989; Greenleaf & Kremen 2006; Blüthgen & Klein 2011). Variation in bee life cycles, nesting habits, foraging ranges, and foraging behaviours can influence the quantity and quality of pollen grains transferred between flowers (Thomson 1986; Hoehn et al. 2008, Pasquet et al. 2008; Fründ et al. 2013), and this functional diversity may explain the high yields achieved by diverse pollinator assemblages. However, it is difficult to discern the mechanisms responsible for enhanced crop pollination by particular bee communities, and the effects of species richness are often confounded with species abundance in studies of crop pollination (e.g. Klein et al. 2003; Hoehn et al. 2008; Rogers et al. 2014).

Research focused on crop pollinator diversity often takes an observational approach, where the existing variation in species richness and abundance within pollinator communities is used to examine associations between pollinator diversity and pollination. Given that these two variables commonly covary, experimental approaches focusing on individual flowers and each individual visitor’s contribution can better disentangle the influence of species richness and abundance on pollination. Further, the link between pollinators and their pollination services is rarely studied directly. Pollinator species richness and abundance are typically measured at the field scale and pollination is measured separately, if at all, as fruit and seed set are often used as a proxies for pollination quality (Klein et al. 2003; Hoehn et al. 2008; Rogers et al. 2014; Mallinger & Gratton 2015). Pollen deposition measurements are traditionally done under a microscope on flowers receiving only one visit by a pollinator (single-visit deposition) or on open-pollinated plants (Reviewed by Ne’eman et al. 2010; King et al. 2013). However, flowers often receive multiple visitors over the period of stigmatic receptivity and the individual visitors’ contributions to the total pollen load are difficult to determine. Due to this methodological difficulty, experiments testing the effects of wild bee individuals and taxonomic groups on pollination and fruit development are scarce (Fontaine et al. 2006; Fründ et al. 2013), especially in field settings. Here, I assert that in order to quantify crop pollination as an ecosystem service in the context of human food production, the pollen contribution of each pollinator must be measured and connected directly to fruit development. This necessitates measurements of the quantity and quality of pollen transferred between crop flowers, to accurately calculate the role of pollinators in food provision. With our heavy reliance on stressed populations of the European honey bee (*Apis mellifera* L.) for the pollination of many commercial crops (Aizen & Harder 2009; Aizen et al. 2019), the influence of wild pollinators on crop yield is a vital area of research.

1.1.4 Bee diversity and the agricultural landscape

An increasing number of field studies that compare pollination services between managed and wild bees are finding that diverse crop pollinator communities can match or exceed the crop production achieved by honey bees alone (Greenleaf & Kremen 2006; Brittain et al. 2013; Garibaldi et al. 2013; Garibaldi et al. 2016). Conversely, reduced landscape complexity and the increase in chemical inputs used to maintain high-yielding crops limits suitable habitat options for wild pollinators in agricultural lands, especially cavity-nesting and ground-dwelling bees (reviewed in Tscharntke et al. 2005). Flowering crops provide temporary floral resources for many pollinators, but a lack of flowering plants outside of the crop blooming period can further reduce the suitability of cropland for many wild bee species (Nicholls & Altieri 2013). When crops are grown within a mix of natural and uncultivated land, bee diversity and crop productivity can be higher than in monocultures or less complex landscapes (Hendrickx et al. 2007; Holzschuh et al. 2008; Ricketts et al. 2008; Carvalheiro et al. 2011). As such, a number of agri-environmental schemes (AES) have been recommended and implemented in Europe and North America to enhance biodiversity in intensified farmlands (reviewed in Wratten et al. 2012) but their degree of efficacy is variable (Kleijn et al. 2006; Scheper et al. 2013). Although not required in Canada, these initiatives promote crop diversification, reductions in pesticide use and tillage, and planting wildflower strips and hedgerows along field margins to increase pollinator resources. The preservation and enhancement of natural and semi-natural areas surrounding crops is also an important practice for restoring wild pollinator services in agricultural landscapes (Kremen et al. 2004; Morandin & Winston 2006; Klein et al. 2012).

Although the composition of the larger landscape surrounding croplands is known to affect the composition of wild bee communities, the influence of elements at the field and farm scale (e.g. field design) on bee diversity has been less explored (but see Kennedy et al. 2013; Fahrig et al. 2015). While it is known that honey bees can travel distances on the order of several kilometres (Visscher & Seeley 1982), the foraging ranges of wild bees are highly variable (Greenleaf et al. 2007). The foraging ranges of small-bodied bees (e.g. *Hylaeus*) can be less than 150 metres (Gathmann & Tscharntke 2002; Zurbuchen et al. 2010), but larger-bodied bees (e.g. *Bombus*) have been known to travel beyond 2 km in search of food (Westphal et al. 2006). If consistent floral resources and habitat options are abundant only at crop edges, small pollinators may be restricted to areas near the crop perimeter (Bartomeus & Winfree 2011; Lander et al. 2011). However, empirical data on the magnitude of pollinator export from field margins to crop interiors is limited (Chacoff & Aizen 2006; Kohler et al. 2008; Morandin and Kremen 2013), especially in temperate agroecosystems.

The composition of crop pollinator communities is also affected by crop management type (e.g. organic vs. conventional), with species richness often declining with management intensity (Rundlöf et al. 2008; Forrest et al. 2015). Thus, the intersection of habitat enhancements with crop management is an important consideration for wild pollinator service provision. Furthering our understanding of the influence of bee diversity on crop production, and the influence of crop production on bee diversity, is an important step towards enhancing pollination services and potentially increasing crop yield in a more sustainable manner.

1.1.5 Strawberry pollination and production

The most widely cultivated strawberry, *Fragaria* × *ananassa* D. (Family: Rosaceae) was developed in the mid-18th century and is a hybrid of the wild strawberry (*F. virginiana*) and the Chilean strawberry (*F. chiloensis*). Strawberry has the 4th highest horticultural farm gate (market) value in Canada, of 128 million dollars, which has been steadily increasing since the early 2000s. Quebec and Ontario are the largest strawberry producers in Canada with 47% and 29% of the national strawberry acreage respectively (AAFC 2017). Most strawberries grown in Canada are June-bearing varieties, which bloom for 14-18 days and produce one large crop, though ever-bearing and day-neutral varieties are growing in popularity (AAFC 2017). Ever-bearing strawberry varieties produce two or three crops throughout the growing season, and day-neutral strawberry varieties (developed from ever-bearers) produce strawberries continually throughout the summer and fall (Bringhurst et al. 1988).

Strawberry plants produce bisexual flowers in determinate inflorescences, each flower having five petals, 20 to 35 stamens, and 100 to 400 pistils. The primary flower is the first to develop on the main branch, and it produces the largest strawberry. The secondary flowers form next in the inflorescence hierarchy, and are approximately 10% smaller than the primary flower. Tertiary and quaternary flowers open thereafter, and produce the smallest fruit (Figure 1.1; Darrow 1966). The strawberry fruit is the product of sexual reproduction, though most commercial varieties are propagated asexually through cloning via stolons. For a fully-formed, marketable strawberry to develop, each of the many ovules within a strawberry flower must be fertilized through pollination to develop into achenes. Unfertilized achenes do not stimulate growth of the berry flesh in the receptacle, and produce misshapen strawberries (Nitsch 1950).

Strawberry pollination is typically achieved through the deposition of allogamous (outcross) and autogamous/geitonogamous (self) pollen by the action of wind, gravity, and insects (Darrow

1966). Flowers typically exhibit protogyny, meaning the stigmas are receptive before anthers dehisce. Many strawberry varieties are self-compatible, but cross-pollinated strawberry flowers often produce larger berries with fewer malformations than flowers receiving self-pollen (Pion et al. 1980; Chagnon et al. 1989); this effect is greater for certain inter-variety pollinations (Colbert & de Oliveira 1992). As such, insect pollination has been shown to increase strawberry yields and reduce malformations by as much as 40% (Free 1968; Pion et al. 1980, Goodman & Oldroyd 1988). However, the influence of pollen type (autogamous vs. allogamous) on fruit development varies across strawberry varieties (Colbert & de Oliveira 1992; Zebrowska 1998).

Wild bees and managed honey bees are the primary strawberry pollinators in Quebec, our study region (Vincent et al. 1990). However, strawberry is not the most attractive crop to honey bees, and flowering plants with concurrent blooms can draw honey bees away from the crop. Wild bee species are beginning to show great potential as effective strawberry pollinators (Klatt et al. 2014; Bartomeus et al. 2014; Horth & Campbell 2018), and some research suggests that wild and managed pollinators exhibit complementarity in strawberry pollination, such that receiving visits from both types of pollinators can increase yield (Chagnon et al. 1993).

Although wild bees show promise as strawberry pollinators, conventional methods of strawberry cultivation may limit wild bee abundance on strawberry farms. Strawberry crops are highly susceptible to insect and mite pests (e.g. flower thrips, aphids, *Lygus* spp., spider mites) and many diseases (e.g. Botrytis Grey Mould, Anthracnose, Powdery Mildew). This makes strawberry one of the most pesticide- and fungicide-laden horticultural crops in North America (USDA, 2017), with 31 chemical pesticides and 48 fungicides registered for use in Canadian strawberry crops (AAFC 2017). The impact of agrochemical residues on strawberry pollinators has been poorly documented but the limited research suggests that complex mixtures of pesticides and fungicides applied in conventionally managed crops increase mortality of pollinating bees (Pilling & Jepson 1993; David et al. 2016; Sgolastra et al. 2016). In conventional strawberry production in Quebec and Ontario, it is also common practice to cover the inter-row area with straw mulch to suppress weeds, keep the strawberries dry during the growing season, and protect the plants in winter. This practice greatly reduces the amount of bare ground within the crop. As most wild bee species are ground-nesting in Canada, mulching likely limits wild bee habitat options within strawberry crops. June-bearing crops remain in production for four to five years so the ground cover remains in place for this period. Day-neutral strawberry crops are usually planted annually and are normally grown in black plastic mulch placed within the row and the application of straw mulch between rows is variable (OMAFRA 2016).

1.1.6 Conclusion

In ecology, and in science in general, our results are only as good as the methods that produce them. Integrating precision methodologies with laboratory and field experiments is integral to the search for general relationships between biodiversity and ecosystem functioning, and in determining how our own activities may be disrupting the very processes we depend upon for survival. Ecological field experiments have been vital in driving BEF research forward, but the vast diversity of life on Earth means that there is still much work to do, especially to further understand the complex links between species across trophic groups.

Bees provide pollination to many of our food, fodder and seed crops, and the relationship between pollinators and plants provides a unique system in which to study biodiversity effects across trophic levels. Despite the importance of bees in agriculture, relatively little is known about the consequences of diversity loss in crop pollinator communities to crop yields. Interactions between bees in diverse bee communities can have facilitative or complementary impacts on pollination and fruit development, which strengthens the argument for biodiversity as a driver of ecosystem functions, but research on these effects is limited to only a few systems (strawberry: Chagnon et al. 1993, sunflower: Greenleaf et al. 2007, pumpkin: Hoehn et al. 2008).

If the conservation of diversity in crop pollinator communities proves to be a viable means to increase crop yield, conserving wild pollinators will become an integral part of sustainable and economical crop management practices. Studies of wild crop pollinators are necessary to determine whether preserving diversity in pollinator communities has the potential to improve crop production in agroecosystems, and to evaluate how agroecosystems can effectively be tailored to preserve pollinator diversity. However, emerging research indicates that few dominant species may provide crop pollination services equal to or better than species-rich bee comminutes (Winfree et al. 2015; Kleijn et al. 2015). Findings such as this serve as a caution against relying too heavily on utilitarian arguments (i.e. ecosystem services) to justify biodiversity conservation and emphasise the importance of arguments based on the intrinsic value of biodiversity.

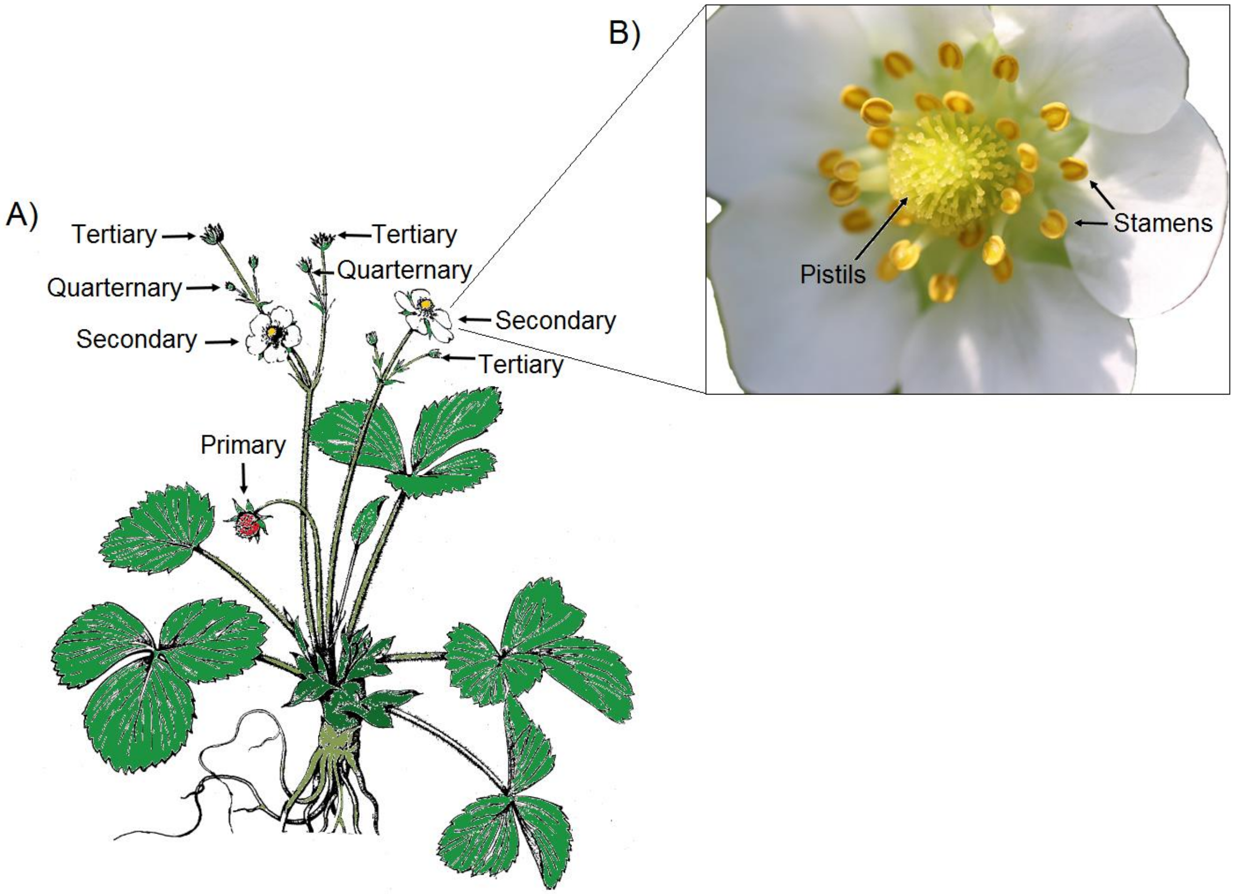

Figure 1.1. A) Strawberry plant inflorescence architecture, showing primary, secondary, tertiary and quaternary flower development. Diagram modified from Darrow (1929). B) Reproductive organs of a strawberry flower. Each pistil has a yellow stigma (to which the pollen adheres), which sits atop a style. Each stamen consists of a pollen-containing anther (yellow) which sits atop a filament (white).

1.2 Thesis objectives

The overarching goal of my research is to examine how bee community composition affects pollination and production in agroecosystems, and how elements of the agricultural landscape impact bee community composition and pollination. My main hypotheses were that 1) species-rich bee communities would improve pollination and production in strawberry agroecosystems through specific, and potentially complementary, foraging behaviours, and 2) larger expanses of natural land surrounding crops would increase species richness and abundance in wild crop-pollinating bee communities, as natural habitat can increase nesting resources and subsequently wild bee spillover into croplands.

Specific Chapter objectives:

The main objective of **Chapter 1** was to develop a technique that would enable direct analyses of the link between pollinators, pollen deposition, and fruit development, and facilitate my investigation of the links between bee community composition and crop production. Through the use of controlled bee diversity field experiments, the goal of **Chapter 2** was to compare the influence of diverse wild bee assemblages to that of managed honey bees on pollination and strawberry production. The main objective of **Chapter 3** was to explore how bee foraging behaviour and field design interact to affect cross-pollination and strawberry yield. The goal of **Chapter 4** was to assess the influence of field-margin habitat on the wild bee community through a survey of the bee communities across large strawberry farms in Eastern Canada.

Connecting text

Before exploring the link between bee community composition and crop pollination, I developed a new pollen deposition measurement technique, and tested its viability by measuring pollen deposition in the flowers of several crop and non-crop plant species with different morphologies. Traditional methods of pollen deposition measurement cannot determine the pollen contribution of each flower visitor in a sequence of visits, and often require removal of the flower from the field, prohibiting direct analysis of fruit yield. In this chapter I describe the 'stigmagraph' technique of pollen measurement, including a detailed protocol for use by other pollination ecologists. This technique uses high-resolution macro photography and image analysis software to calculate the pollen contribution of each flower visitor in a sequence of visits, and allows the flower to remain intact in the field for direct fruit analyses. The development this pollen measurement technique was essential to proceed with my investigation of the influence of bee community composition on pollination.

Author contributions: The authors of this work are Gail MacInnis[1] and Jessica Forrest[2]. GM conceived the ideas and designed the study. GM conducted proof-of-concept tests, collected the data, conducted analyses, and wrote the manuscript. JF had input on experimental design, and provided critical contributions to the manuscript drafts.

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Chapter 2: Quantifying pollen deposition with macro photography and ‘stigmagraphs’

2.1 Abstract

The pollen deposited during a single visit by a flower visitor (“single-visit deposition”; SVD) is often measured by removing the stigma from the flower and counting the pollen grains deposited under a microscope. This process precludes study of any subsequent interactions between the flower and later visitors (such as pollen removal from the stigma). Furthermore, if the stigma is excised too soon after the pollinator visit, the flower may be rendered infertile, such that any analyses of fruit or seed yield in relation to pollen deposition must be done indirectly. Here, a method of pollen deposition measurement was developed using macro photography and the open-source image-analysis software program ImageJ/Fiji. Using colour segmentation options within the program, the pollen grains can be distinguished from the background stigmatic surface, and the percentage of stigma coverage can be calculated. This pollen deposition measurement method leaves the sampled flower in the field to develop into fruit, allowing any subsequent yield or quality analyses to be conducted directly.

2.2 Introduction

Experiments in pollination ecology often require a measure of the amount of pollen deposited onto a flower’s stigma. A measure of pollen deposition is required in order to compare pollen-transfer and pollen-deposition efficiencies among different species of flower visitors (Schemske & Horvitz 1984; Motten et al. 1981; Javorek et al. 2002; Adler & Irwin 2006; Garibaldi et al. 2013) or simply to distinguish between flower visitors and true pollinators (King et al. 2013). Pollen deposition measurements are also used when assessing the influence of pollen quantity on fruit and seed set in wild and agricultural plants (Falque et al. 1995; Waites & Ågren 2004; Ne’eman et al. 2010).

It is difficult to measure the pollen load on a flower’s stigma due to the small size of most stigmas, the vast number of pollen grains that can be deposited, and the potential to dislodge pollen during the measurement process. Further, because of the three-dimensional nature of many stigmas, pollen can be deposited in several focal planes, making the grains difficult to count (Flanagan et al. 2009). A widely used technique to determine the pollen load on floral stigmas is the stigma squash method (Kearns & Inouye 1993, Dafni et al. 2005, section 3.10). The stigma squash relies on a visual count of the pollen grains on a flower's stigma under a microscope. Counting pollen on a squashed stigma can be a time-consuming process, and subsampling is often required when thousands of pollen grains have been deposited on a single stigma. A stigma squash also requires the flower's stigma to be removed from the field. If a flower's stigma is removed too soon, i.e. prior to pollen-tube growth and ovule fertilization, analyses of seed or fruit production by the same flower are not possible (Faegri & Van der Pijl 1979; Kearns & Inouye 1993). Lastly, a flower is usually visited by multiple pollinators, and pollen counts determined through a stigma squash cannot determine each individual species' contribution to the total stigmatic pollen load.

The purpose of the present study was to develop a pollen-deposition measurement technique that could be used to accurately quantify pollen deposition on stigmas, partition the stigmatic pollen load by pollen vector, and allow the sampled flower to remain in the field to develop fruit and seed. These particular criteria were chosen as they are important for research in pollination ecology and for studies relating pollination to fruit and seed development. Image analysis methods have successfully been used to count the number of pollen grains within flower anthers and on stigmas in a laboratory setting (Costa & Yang 2009), but had not been tested on flower stigmas that are to remain in the field. The 'stigmagraph' method of pollen deposition measurement developed here uses macro photography and image-analysis software to measure pollen deposition *in situ*. A stigmagraph is a photograph of a flower's stigma, taken in the field and subsequently analyzed to determine the pollen count. Two species of daffodil (*Narcissus pseudonarcissus* and *Narcissus nanus*) were used to test and compare the stigmagraph technique to the standard stigma-squash technique. Pumpkin (*Cucurbita pepo* L.) flowers were used to determine if individual pollen grains on larger stigmas could be counted using the stigmagraph method. Pollen deposition was also quantified on strawberry stigmas (*Fragaria* × *ananassa*) using the stigmagraph technique and compared to the seed set of the developed fruit.

2.3 Methods

2.3.1 Stigma photography

First, a photo is taken of the virgin stigma of the sample flower before any flower visits have taken place. This is done to determine the baseline amount of pollen (if any) deposited on the stigma prior to any flower visits, and to accurately determine the amount of pollen deposited by each visitor thereafter. A photograph (hereafter a ‘stigmagraph’) of the sample flower’s stigma(s) is then taken after each flower visit. Each stigmagraph needs to be taken at the same angle, lighting, and camera aperture setting to ensure consistency for image analysis. For a stigmagraph to be adequate for image analysis, it must be taken with a camera fitted with a dedicated, macro lens (at least 1:1 magnification). The type of lens used will depend on the size of the stigmas of the sample flower and the working distance required. A standard macro lens, such as a Canon EF 100 mm macro lens, is adequate for flowers with a pistil surface of greater than 2 mm in diameter. Macro photography inherently involves working with a very shallow depth-of-field (DOF). To achieve the largest depth-of-field possible, the photos should be taken with a small aperture opening (f/16). Taking the photographs further away from the flower will increase DOF, but will sacrifice image detail. This is undesirable for the subsequent photo analysis and detection of pollen grains. If the stigmatic surface is not flat, as is often the case, it is best to capture a series of images of the stigma surfaces at increasing focal depths. Focus stacking, or focal plane merging, can then be performed on the images at the processing stage (Johnson 2008).

Once the photos of the stigma(s) are captured in the field, the photos are processed through ImageJ/Fiji to determine the amount of pollen deposited on the stigma. ImageJ is a free, Java-based image-processing program and Fiji is an open-source image-processing package which operates within the ImageJ software environment (Schneider et al. 2012; Schindelin et al. 2012). For flowers with simple morphology, such as the single-pistiled daffodil (*Narcissus* spp.), pollen grains can be distinguished from the background stigmatic surface using the segmentation options within ImageJ, namely colour thresholding. Two *Narcissus* spp. were used here to test the stigmagraph method with two different-sized pollen grains, and because they were readily available in the field.

Photographs of several pumpkin (*Cucurbita pepo* L.) stigmas were taken after a series of flower visits to determine if the number of pollen grains deposited on larger stigmas could be counted in a set of focus-stacked photos using the Fiji Cell Counter plugin. By adjusting the camera angles, the full extent of the stigmatic surface can be captured, but this requires time and manipulation of floral structures. As such, the pumpkin stigmas were only photographed from the top down; the underside of the stigmas was not captured in the stigmagraphs.

For more complex flowers, such as the multi-pistiled strawberry (*Fragaria* spp.), the Trainable Weka Segmentation application (Hall et al. 2009) is more suitable to determine the total stigmatic pollen load. Alternatively, on a flower with multiple pistils, the number of stigmas carrying pollen can be counted from the photographs using Cell Counter. This is the simplest option for a researcher requiring a simple measure of the number of stigmas having received pollen. Strawberry was chosen as a test flower because of the multi-pistiled morphology and the ability to assess successful pollination from the outside of the fruit. The three photo-processing options used to calculate the proportion of the stigmatic surface covered by pollen—ImageJ Colour Thresholding, the Trainable Weka Segmentation and manual counting with Fiji's Cell Counter—are described in detail below.

2.3.2 Measuring pollen deposition with ImageJ Colour Thresholding

The proportion of the stigmatic surface covered by pollen can be calculated using ImageJ Colour Thresholding (ICT) as follows:

1. Open the image of the virgin stigma in Fiji. Convert the image to a RGB color photograph under 'Image' in the *Type* menu.
2. To remove the background, use the 'Adjust color threshold' option under the *Image>Adjust* menu. Select the 'Dark background' option to fill the stigmatic surface in black (Fig. 2.1A). Click the 'Select' button, and close the *Color Threshold Window*. Then select 'Clear outside' under the *Edit* menu. This will remove the background and leave only the stigmatic surface in the photo. Select 'Binary' under the *Process* menu and select 'Make binary'. This will create an image similar to Fig. 2.1A.

3. Select ‘Histogram’ under the *Process* menu and then select ‘List’. This will open a table of values. The first column of the table contains a list of colour values; the second column contains the area of each colour, in pixels. The area corresponding to colour-value 255 (black) is the area of the stigmatic surface.

Note: To measure the pollen deposition in terms of proportion of stigmatic area, a scale is not needed. If a measurement of pollen deposited in mm^2 or cm^2 is desired, a scale can be set under the *Analyze* menu. Only measurements of the proportion of the stigma surface covered in pollen are described here.

4. Open the next image of the sample stigma, taken after the flower has received a visit. Repeat step 1, and use the ‘Adjust color threshold’ option under the *Image>Adjust* menu and de-select the ‘Dark background option’. Then click ‘Select’ and close the *Color Threshold Window*. Then select ‘Clear’ (not ‘Clear Outside’) under the *Edit* menu.
5. Open the *Color Threshold Window*. Move the sliders on the ‘Hue’, ‘Saturation’, and ‘Brightness’ options until only the pollen is highlighted in red. This effectively allows only those colours associated with pollen to pass and thus segments the photo into pollen and non-pollen areas (Fig. 2.1C).
6. Select ‘Binary’ under the *Process* menu and select ‘Make binary’. This will create an image similar to Fig. 2.1D. Select ‘Histogram’ under the *Process* menu and then select ‘List’. This will open a table of values. The value next to 255 in the table is the area (in pixels) of the stigmatic surface covered by pollen.
7. Divide the number of black pixels obtained in Step 6 by the number of pixels obtained in Step 3 to obtain the proportion of the stigma covered by pollen.

2.3.3 Measuring pollen deposition with Trainable Weka Segmentation

For more flowers of more complex morphology, the proportion of the stigmatic surface covered by pollen can be calculated using the Trainable Weka Segmentation (TWS) application, as follows:

1. TWS is a tool built into the Fiji version of ImageJ which can be accessed through the ‘Segmentation’ option in the *Plugins* menu.
2. Once in the TWS environment, zoom in on the stigmagraph(s) as much as possible. Use the *Freehand selection* tool to manually select areas of the photograph that contain pollen and pick ‘Add to Class 1.’ Once 2 or more areas clearly containing pollen are selected, select areas that do not contain pollen and add them to ‘Class 2’. Then click *Train classifier*. This trains the application to define colours in Class 1 pixels as pollen, and Class 2 pixels as non-pollen (Fig. 2.2A).
3. Once the classifier training is finished (this can take a while, depending on computational power), select *Create result* in the left-hand menu. This will create an output similar to Fig. 2.2B.
4. Convert the image to binary (Fig. 2.2C) and obtain a histogram of colour values, as described in Step 6 of the ImageJ Colour Thresholding section (above). The amount of pollen in the photo is the number of black pixels indicated in the histogram table.

2.3.4 Measuring pollen deposition with Cell Counter

The Fiji Cell Counter can be used to count and track particles or objects in an image. Cell Counter can be used to count stigmas containing pollen as follows:

1. Select the *Cell Counter* plug-in under *Plugins* in the *Analyze* menu.
2. Initialize the photo by clicking the initialize button in the *Cell Counter* window.
3. Rename *Type 1* and *Type 2* to “Pollen” and “No Pollen”, respectively (Fig. 2.3B).
4. Select *Type 1* and use the *Arrow* tool to click on the stigmas that contain pollen grains.
5. Select *Type 2* and use the *Arrow* tool to click on the stigmas that do not contain pollen grains.
6. Select *Results* to obtain a table containing the number of stigmas with and without pollen.

2.3.5 Comparing the stigmagraph method to the stigma-squash method

To test the ICT and the TWS measurements against the standard stigma-squash technique, 11 flowers of *Narcissus pseudonarcissus* and 11 flowers of *Narcissus nanus* were hand-pollinated by lightly touching a dehiscing *Narcissus* anther to each stigma. The stigmas were then removed and photographed. In each photo, the proportion of the stigma covered in pollen was determined (in pixels) by both ICT and TWS. This proportional area of pollen on the stigma surfaces was then divided by the surface area of one *Narcissus* pollen grain to estimate the number of pollen grains on the stigma. The ICT method was performed twice on each stigma - once by an experienced stigmagrapher and once by an observer new to the stigmagraph method, in order to compare the processing time between users. The TWS measurement was only conducted once due to limited computational power. The time it took to process each stigmagraph with ICT was recorded for 12 stigmagraphs (6 *N. nanus* and 6 *N. pseudonarcissus*). The timing of the ICT method began when the photos were transferred to the computer and open in ImageJ, and ended when the user determined the number of pollen grains on the stigma.

Once photo processing was completed, the stigmas were squashed on microscope slides with fuchsin-stained gelatin. The pollen grains were counted on each stigma at 400 × magnification once by each observer, without sub-sampling. For 12 stigma-squash slides (6 *N. nanus* and 6 *N.pseudonarcissus*), the time required to complete the pollen counts on the stigma squash was recorded for each observer. The stigma-squash timing began once the stigma was mounted on the slide and ready to be counted.

2.3.6 Comparing stigmagraph pollen counts to seed set

In the spring of 2015, 120 commercial strawberry plants (*Fragaria × ananassa, "*Jewel" variety) were haphazardly selected at the McGill Horticultural Research Farm, Montreal, QC (45.4067°,−73.9401°) for a comparison of the number of stigmas containing pollen, as calculated using stigmagraphs, and seed set. A strawberry flower contains many carpels, each of which contains one ovary and one ovule. Once successful fertilization occurs, the ovaries of the strawberry flower develop into one-seeded achenes. Hereafter, seed set refers to the number of fertilized achenes per strawberry. The development of achenes is what stimulates the growth of the edible tissue of the receptacle (Nitsch 1950). Thus, the weight and shape of the berry is directly related to the number of achenes on a strawberry, which largely depends on the number of ovules successfully fertilized through pollination.

The selected strawberry plants were covered with cotton mesh at the bud stage and one flower of each plant was labelled. Once flowers were open, the mesh was removed to allow pollinators to visit and the flowers were photographed after each pollinator visit. To vary the quantity of pollen deposited, flowers were exposed to a varying number of pollinator visits (1–10); mesh was then replaced over the flowers. The strawberry variety used in this study was protogynous and the sampled flowers were not emasculated, so the flower stigmas were also photographed two days later to capture changes in the pollen load due to autogamous pollen deposition. After all photographs had been taken, the strawberry plants were kept covered until the stigmas began to turn brown and the petals had abscised.

The total number of stigmas with pollen (including any autogamously deposited pollen) was calculated using the Fiji Cell Counter on the stigmagraphs taken at the end of receptivity, as described above. Once the fruit had developed, each strawberry was harvested and cut in half, and each side was photographed to avoid double-counting any achenes. Achene diameters were determined from the berry photos using the ImageJ *Measure* function. As in Ariza et al. (2012), any achene ≤ 0.4 mm in diameter was considered unfertilized (Fig. 2.4). Fertilized achenes were counted using Fiji's Cell Counter.

2.3.7 Data analysis

The differences in pollen counts between the stigma-squash and stigmagraph methods were normally distributed, so paired t-tests were used to compare the pollen load of the daffodil stigmas as determined by each stigmagraph analysis method (ICT and TWS) and the pollen load determined using the stigma-squash method. The pollen counts for each observer were first analysed separately; i.e., the ICT pollen counts done by one observer were compared to the stigma-squash counts of the same observer; we then compared the two methods using the pooled data from both observers. The pooled data were log-transformed to meet the assumption of multivariate normality (tested by Royston's Multivariate Normality test) and the relationship between stigmagraph pollen counts and stigma-squash pollen counts were assessed through linear regression. The TWS was only done once by the experienced observer and was not included in the pooled analysis. The timed ICT counts and stigma-squash counts of each observer were compared using paired t-tests.

The relationship between strawberry seed set and number of pollen-bearing stigmas (as determined by Cell Counter) was modeled with simple linear regression. The number of fertilized achenes was expected to be less than (if resources were limiting) or equal to the number of stigmas bearing pollen. A paired t-test was used to compare the number of pollen-bearing stigmas to the number of seeds per strawberry.

2.4 Results

The average time to process a photo of a daffodil stigma with ImageJ Colour Thresholding (ICT) and determine the pollen load was 11.7 ± 4.4 minutes (mean ± s.d.) for an experienced stigmagrapher. The average time for the same individual to count the daffodil pollen grains on a squashed stigma was not significantly different, at 11.9 ± 3.6 minutes ($t(11)=0.17$, $p = 0.89$). The average time for an individual new to the stigmagraph technique to photograph a stigma and process the photo was 20.2 ± 7.1 minutes, vs. only 13.42 ± 4.79 minutes for a stigma-squash pollen count ($t(11) = 4.45$, $p < 0.001$). Thus, the inexperienced observer took significantly longer than the experienced observer to process a stigmagraph with ICT ($t(11) = 2.96$, $p = 0.01$), whereas there was no significant difference in stigma-squash counting time between observers ($t(11) = 0.73$, $p = 0.24$). The average time to count pollen-bearing stigmas on a strawberry flower stigmagraph was 5.8 ± 2.1 minutes. The latter procedure was only conducted by one observer.

The stigmagraph and stigma-squash methods yielded similar stigmatic pollen counts (Table 2.1, Fig. 2.5). The ICT method tended to estimate a higher number of pollen grains per stigma than the stigma-squash method for both observers, but this difference was non-significant for the experienced observer and only marginally significant for the inexperienced observer (Table 2.1). The inexperienced observer's ICT pollen counts were, on average, significantly higher than those of the experienced observer (Table 2.1).

The relationship between the pollen deposited and seed set for strawberry was analysed for 95 flowers; 25 berries were lost before harvest. There was a positive linear relationship between the number of stigmas pollinated on strawberry flowers and the number of seeds produced by those flowers (Fig. 2.6). The number of seeds per flower was not significantly different from the number of pollen-bearing stigmas, as determined by Cell Counter ($t(94) = 1.11$, $p = 0.27$), but 44% of the flowers had fewer pollen-bearing stigmas than developed seeds.

For the stigmagraph test of the large stigmas of pumpkin flowers, the pollen grains were easily recognizable in the stigmagraph (Figure 2.7A) and readily counted using Fiji's Cell Counter (Figure 2.7B). Although the photographing time was not recorded, it certainly takes more time to photograph the entire surface a large, multi-lobed stigma like that of pumpkin, than smaller or less-complex stigmas.

2.5 Discussion

The stigmagraph method produces stigmatic pollen deposition measurements similar to those of the traditional stigma-squash method. The stigmagraph method does not necessarily reduce pollen-counting time compared to the stigma-squash method, but this new method is certainly a feasible way to determine pollen load when preparing a stigma squash is not possible—for instance, if stigmas are too large (e.g., Cucurbitaceae spp., *Lilium* spp.) or too numerous (e.g., *Fragaria* spp.). Pollen grains on large stigmas can be counted individually by taking a series of photographs focused throughout the depth of the stigmas. The underside or obstructed sections of a stigma can be captured by adjusting the camera angle or removing the petals. However, manipulating floral structures may affect the behavior of flower visitors and the problem of capturing pollen grains that are completely masked by other grains will remain regardless of camera angle. Further testing of the stigmagraph method is needed to test the accuracy of this pollen-counting technique for stigmas of variable complexity. The stigmagraph method may prove capable of quantifying pollen deposition on flowers of many other plant families, such as Cactaceae, Iridaceae, or in orchids lacking pollinia (e.g. *Cypripedium* spp.).

The time required to process a stigmagraph and the accuracy of the pollen count is highly dependent upon user experience. Once a researcher becomes experienced with the ImageJ software environment, the time it takes to calculate pollen load can be less than that of a stigma squash, especially if the stigma contains a large number of grains. The accuracy of the stigmagraph method depends on the observer's ability to capture focused, high-resolution photographs of the sample flowers, which requires some practice. If the image contains aberrations due to the camera flash or light reflections, it is harder to correctly distinguish pollen from background surfaces during image processing. The 'Remove outliers' function is one option within ImageJ that can be used to reduce reflection effects (see Ferreira & Rasband 2012), but it is preferable to avoid image irregularities at the photo capture stage. Once the photography portion is mastered, this method is quick and suitable for field situations.

The accuracy of the stigmagraph method also depends on the user's ability to correctly define what constitutes pollen on the photos of the stigmas. This subjectivity introduces the potential for measurement bias, and for within- and between-observer variation in pollen counts. This subjectivity can be reduced by using the TWS tool, but if the illumination varies across stigmagraphs, pollen can be erroneously selected by the TWS as well. However, there is no reason to believe that error associated with the stigmagraph method would be more than that of the stigma-squash method, as the stigma squash involves manually counting pollen grains under a microscope, subsampling, or using similar image analysis software to determine pollen loads.

A problem with both the stigmagraph and stigma-squash methods is that pollen grains often clump together and can be deposited in several layers. Only the topmost layer of pollen can be seen in a stigmagraph. Squashing a stigma may redistribute the grains in a more uniform plane, but there can often still be a great deal of pollen clumping on a stigma-squash slide. Surprisingly, the pollen load determined on the daffodil stigmagraphs here was usually higher than that of the pollen counts on the stigma squashes. This discrepancy was most likely due to some non-pollen image aberrations being classified as pollen by the ICT. It was noticed that the reflection of light in the secreting papillae of some of the daffodil flowers was often classified as pollen by the ICT and the TWS methods. This was corrected for in most of the ICT and TWS iterations done by the experienced observer, but not by the inexperienced observer for the ICT counts.

A 1:1 relationship was expected between the number of pollen-bearing stigmas and the seed set of strawberry, but some variation is to be expected due to post-pollination processes such as ovule abortion. Although seed set is largely dependent on successful pollen deposition, the viability and successful germination of the deposited pollen can be affected by climate and environmental factors (Žebrowska 1995; Ledesma & Sugiyama 2005). Florivorous insects can also damage reproductive organs post pollination (Howitt et al. 1965; Ariza et al. 2012). These factors may explain some of the variation in the number of stigmas pollinated versus seed set, as western flower thrips (*Frankliniella occidentalis*) and tarnished plant bugs (*Lygus lineolaris*) were observed on the sampled flowers. However, the intercept of the regression of seed set on pollen-bearing stigmas was positive, indicating that more seeds were produced than would be expected based on pollen deposition (at least at low levels of pollen deposition). The higher number of seeds than pollen was most likely due to autogamous pollen that was deposited after the stigmagraphs were taken, as strawberry is self-compatible and the stigmas can remain receptive for 3-7 days (McGregor 1976). The flowers were not emasculated and final stigmagraphs were taken two days after the flower visits, so it can be assumed that the anthers of some of the sampled flowers still contained pollen.

There are several other limitations to consider when attempting to use the stigmagraph method for pollen deposition measurement, namely, the colour of the pollen grains, the size of the stigmas, and computational power. Segmenting the photos can be difficult (and sometimes impossible) when the colour of the pollen is similar to the colour of the stigmatic surface. It can take a great deal of adjusting and readjusting to reach the correct threshold values using ICT (described above). The TWS readjusts automatically but it requires time to train the classifier on complex photos. The photos needed for this method are intrinsically large and the time required to complete the TWS on an image is highly dependent on computational power. It is recommended that 16 gigabytes or more of memory be dedicated to ImageJ to hasten photo processing. Several test stigmagraphs should be taken and processed to ensure that pollen can be distinguished from the background of the stigma prior to using this method for research purposes.

The stigmagraph method is currently not suitable for flowers with tiny reproductive structures. It was not possible with the camera lens used in this study (Canon© EF 100 mm macro) to obtain images suitable for analysis of very small stigmatic surfaces. The smallest stigmas successfully tested here were those of commercial strawberry, which are approximately 0.8 mm in diameter. Further testing with lenses of higher magnification capabilities is required to determine if the stigmagraph method can be used to measure pollen deposition on minuscule stigmas. It is presently more advisable to use the traditional stigma squash technique to determine the pollen load on stigmas smaller than 0.8 mm. For stigmas of larger size and depth, it would be necessary to use a focus-stacked set of photos to capture the majority of the stigmatic surface. Focus-stacking requires more time at each flower in the field but does not add much time to the image-processing stage. Fiji and ImageJ are well equipped to deal with photo stacks (see Ferreira & Rasband 2012). Lastly, the stigmagraph method partitions the pollen deposited by the colour of the pollen grains. If many different co-flowering plant species are present in an area, a stigmagraph cannot discern the species identity of the deposited pollen grains, or whether or not the deposited pollen is viable.

Nonetheless, the stigmagraph method is a viable means of measuring stigma pollen load and was successfully used here to obtain pollen counts similar to those of the stigma squash method. Most notably, the stigmagraph method allows the sampled flower to remain in the field without manipulation to develop into fruit and seed. This enables a direct comparison of pollinator performance to fruit and seed set, without manipulating the sampled flower. Further, measurements of the pollen deposited by multiple successive flower visitors can be obtained using this technique, allowing the determination of each species' contribution to the stigmatic pollen load. The stigmagraph method also has the potential to track the location of pollen placement on flower stigmas, and the rate of pollen removal from stigmatic surfaces, especially in flowers with larger stigmas and pollen grains. Thus, this new method of pollen deposition measurement has strong potential to advance the field of pollination ecology.

Table 2.1. Comparison of the pollen counts determined by the stigmagraph processing methods (ImageJ Colour Thresholding – ICT, Trainable Weka Segmentation - TWS) and the pollen counts on the stigma squash slides for an experienced and an inexperienced observer. A positive mean difference indicates that the first method listed estimated a higher number of grains than the second method.

Observer	Compared methods	Mean difference	t	df	p
Experienced	ICT vs. Stigma squash	0.7%	0.61	21	0.55
Inexperienced	ICT vs. Stigma squash	4.0%	1.96	21	0.06
Experienced	TWS vs. Stigma squash	2.1%	1.07	21	0.30
Both (pooled)	ICT vs. Stigma squash	2.3%	1.93	43	0.06

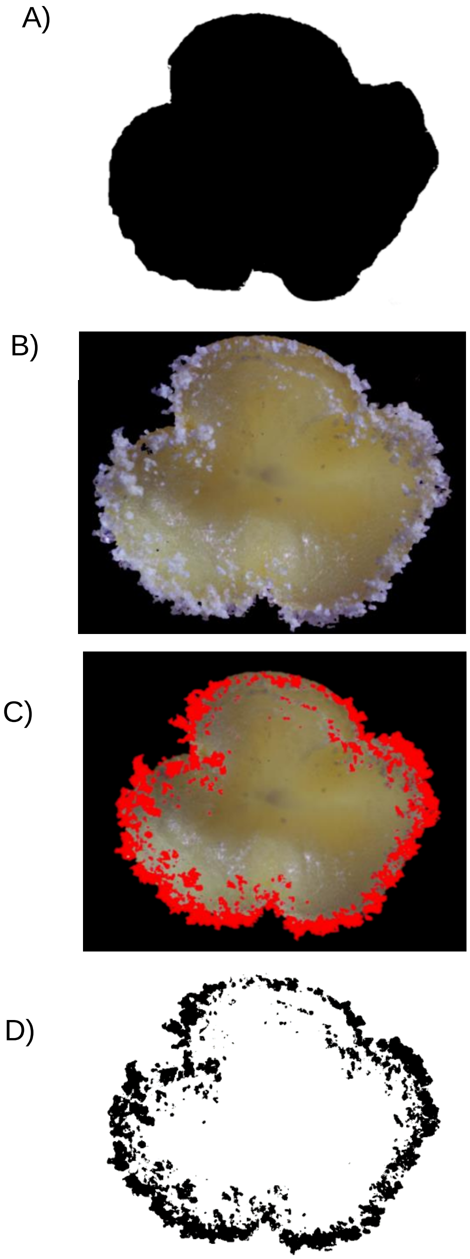


Figure 2.1. Segmenting the pollen-deposition photographs with the ImageJ Colour Thresholding (ICT) method. A) Binary photo of a virgin daffodil stigma (*Narcissus pseudonarcissus*). B) The same stigma with background removed. C) The segmented photograph, with pollen grains selected using colour thresholding. D) The binary photo of pollen deposition used to create a histogram and quantify the proportion of the stigmatic surface covered by pollen.

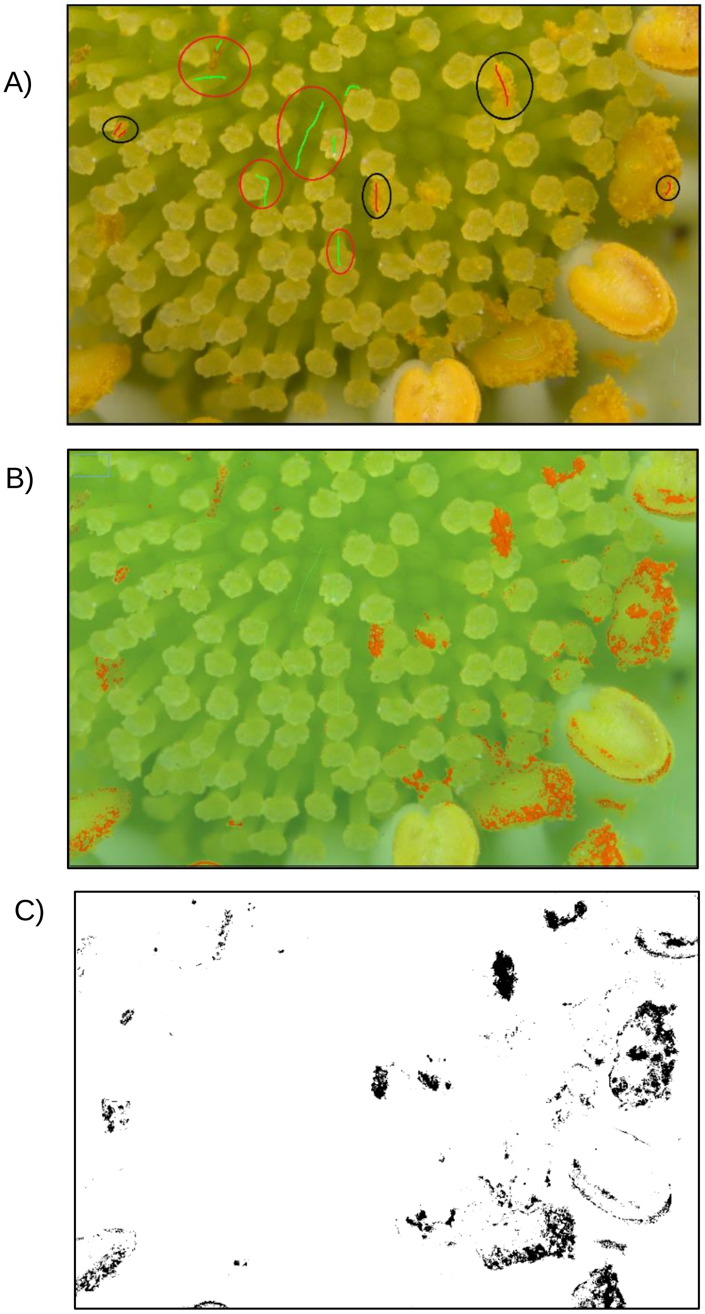
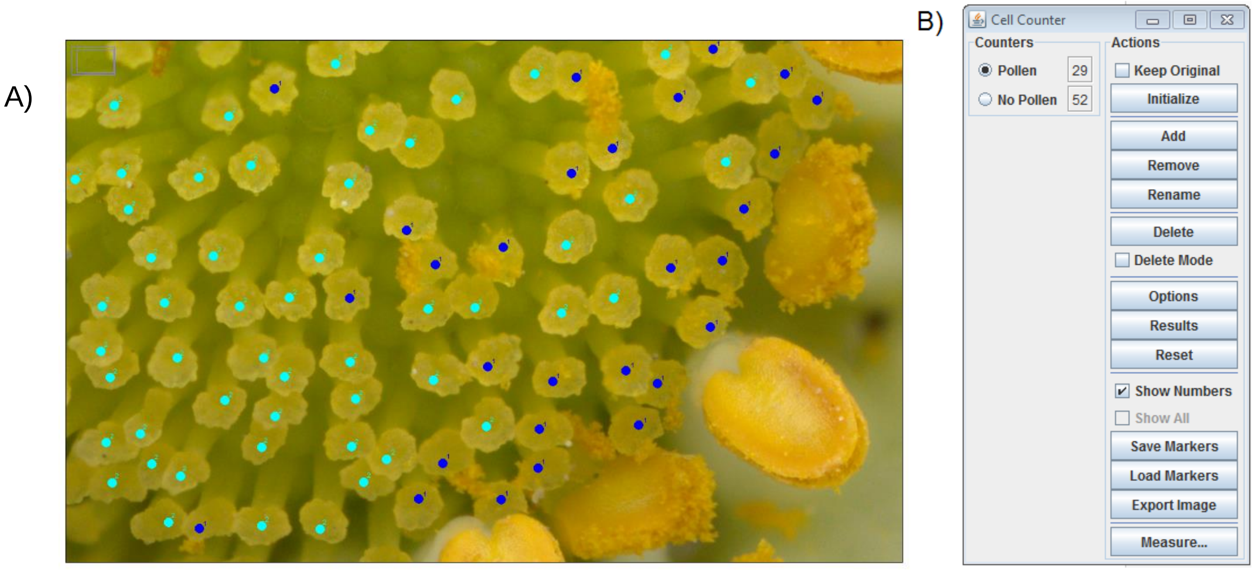


Figure 2.2. Section of macro photograph of strawberry (*Fragaria* x *ananassa* D.) stigmas and several anthers after processing through the Trainable Weka Segmentation (TWS) application in ImageJ. A) The TWS training window; red lines are the classification selections for pollen (circled in black), and green lines are the classification selections for non-pollen (circled in red). B) The output of the TWS. The orange areas indicate sections classified as pollen. C) The binary image used for the total pollen deposition calculation.



● Pollen 29
○ No Pollen 52
☐ Keep Original
☐ Delete Mode
☑ Show Numbers
☐ Show All

Figure 2.3. Counting stigmas containing pollen grains with Cell Counter in Fiji. A) Strawberry (*Fragaria* x *ananassa* D.) stigmas with pollen (blue) and without pollen (green) selected using the Cell Counter tool. B) The Cell Counter menu.

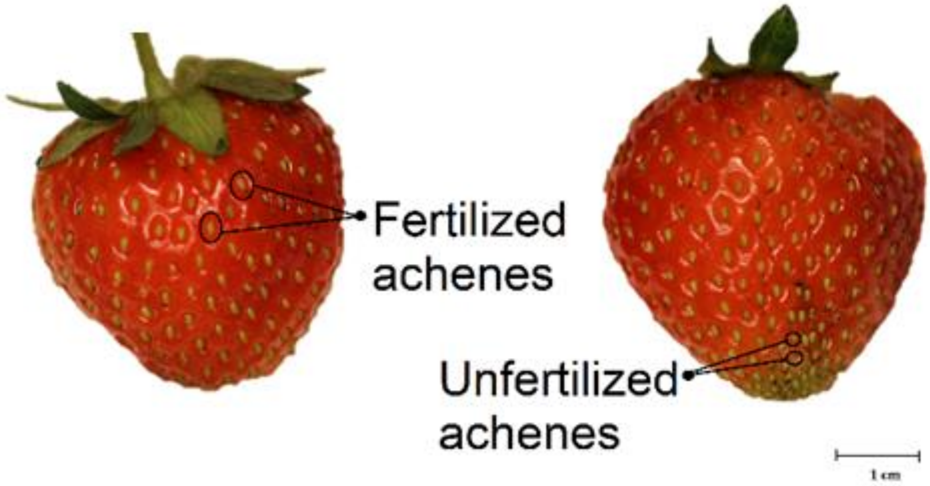


Figure 2.4. Fertilized and unfertilized achenes of a sampled strawberry. Fertilized achenes are those with a diameter > 0.4 mm and unfertilized achenes are those with diameter ≤ 0.4mm.

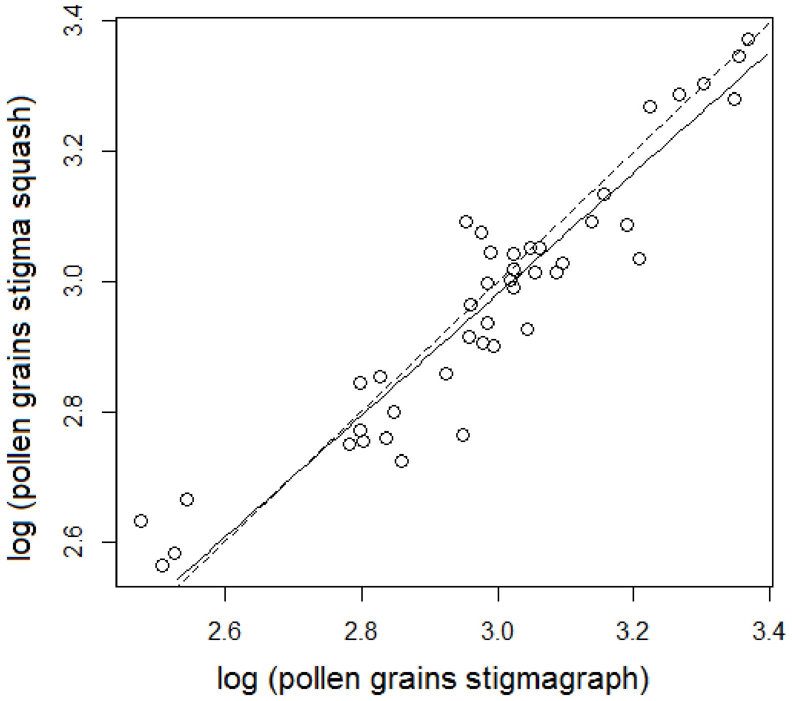


Figure 2.5. The relationship between the average number of pollen grains determined by the ImageJ Colour Thresholding (ICT) method in stigmagraphs and the average number of pollen grains determined by stigma squashes for two species of daffodil (*N. pseudonarcissus* and *N. nanus*). The SMA regression line (solid) has slope = 0.93 ± 0.09, i.e. not significantly different from 1, and intercept = 0.18 ± 0.3, i.e. not significantly different from 0. The dotted line is the 1:1 line. N=44 observations of 22 stigmas; R^2=0.88.

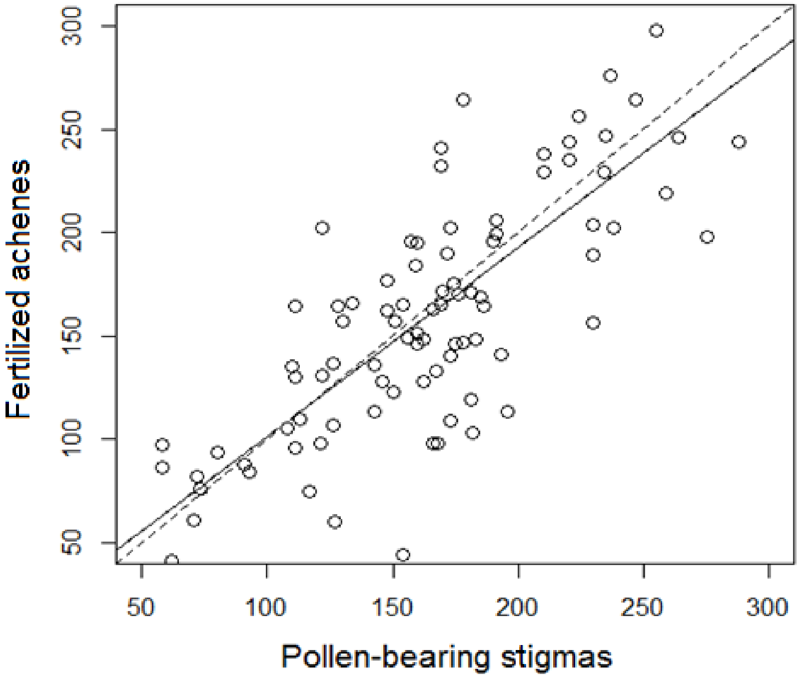


Figure 2.6. The relationship between number of seeds per flower and number of pollen-bearing stigmas as calculated by stigmagraphs for commercial strawberries (*Fragaria* x *ananassa* D.). The regression line (solid) has slope = 0.92 ± 0.15, i.e. not significantly different from 1, and intercept = 9.25 ± 26.2, i.e. not significantly different from 0. The dotted line is the 1:1 line. N=95 flowers; R^2=0.61.

Figure 2.7. Counting pollen on larger stigmas with stigmagraphs. A) A focus-stacked stigmagraph of the multi-lobed stigma of a pumpkin flower (*Cucurbita pepo* L.). B) A section of the stigmatic surface with the individual pollen grains counted using Fiji's Cell counter.

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2.7 References

Adler, L.S., Irwin, R.E., 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Ann. Bot. 97(1), 141-150.

Ariza, M.T., Soria C., Medina-Mínguez J.J., Martínez-Ferri, E., 2012. Incidence of misshapen fruits in strawberry plants grown under tunnels is affected by cultivar, planting date, pollination, and low temperatures. HortScience. 47(11), 1569-1573.

Conner, J.K., Davis R., Rush S., 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. Oecologia 104(2), 234-245.

Costa, C.M., Yang, S., 2009. Counting pollen grains using readily available, free image processing and analysis software. Ann. Bot. 104(5), 1005-1010.

Cresswell, J.E., 2000. Manipulation of female architecture in flowers reveals a narrow optimum for pollen deposition. Ecology 81(11), 3244-3249.

Dafni A., Kevan, PG, & Husband, BC, 2005. *Practical Pollination Biology*. Cambridge, Ontario, Canada. Enviroquest, Ltd.

Falque, M., Vincent, A., Vaissiere, B., Eskes, A., 1995. Effect of pollination intensity on fruit and seed set in cacao (*Theobroma cacao* L.). Sex. Plant Reprod. 8(6), 354-360.

Ferreira, T., Rasband, W., 2012. ImageJ User Guide - ImageJ/Fiji 1.46r. URL: https://imagej.nih.gov/ij/docs/ guide/user-guide.pdf (accessed April 2015).

Flanagan, R.J., Mitchell, R.J., Knutowski, D., Karron, J.D., 2009. Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). Am. J. Bot. 96(4), 809-815.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., […], Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127), 1608–1611.

Hall, M., Frank, E., Holmes, G., Pfahringer, B., Reutemann, P., Witten, I.H., 2009. The WEKA Data Mining Software: An Update. ACM SIGKDD Explorations Newsletter 11.1.

Harder, L.D., Thomson, J.D., Cruzan, M.B., Unnasch, R.S., 1985. Sexual reproduction and variation in floral morphology in an ephemeral vernal lily, *Eyythronium Americanum*. Oecologia 67(2), 286-291.

Howitt, A.J., Pshea, A., Carpenter, W.S., 1965. Causes of deformity in strawberries evaluated in a plant bug control study. Michigan Agricultural Experiment Station Quarterly Bulletin 48, 161-166.

Javorek, S.K., Mackenzie, K.E., Vander Kloet, S.P. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Ann. Entomol. Soc. Am. 95(3), 345-351.

Johnson, D., 2008. *How to Do Everything: Digital Camera, 5th edition*. McGraw-Hill Osborne Media, New York.

Kearns, C.A. Inouye, D.W., 1993. *Techniques for Pollination Biologists***.** University Press, Colorado.

King, C., Ballantyne, G., Willmer, P.G., 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. Methods Ecol. Evol. 4(9), 811-818.

Ledesma, N., Sugiyama, N., 2005. Pollen quality and performance in strawberry plants exposed to high-temperature stress. J. Am. Soc. Hortic. Sci. 130(3), 341-347.

McGregor, S.E., 1976. Insect pollination of cultivated crop plants (Vol. 496). Agricultural Research Service, US Department of Agriculture.

Motten, A.F., Campbell, D.R., Alexander, D.E., Miller, H.L., 1981. Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica.* Ecology 62(5), 1278-1287.

Ne'eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S.G., Dafni, A., 2010. A framework for comparing pollinator performance: effectiveness and efficiency. Biol. Rev. 85(3), 435-451.

Nitsch, J.P., 1950. Growth and morphogenesis of the strawberry as related to auxin. Am. J. Bot. 37(3), 211-215.

Schemske, D.W., Horvitz, C.C., 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225(4661), 519–521.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. 9(7), 671–675.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., […], Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Method. 9(7), 676-682.

Schlindwein, C., Wittmann, D., Martins, C.F., Hamm, A., Siqueira, J.A., Schiffler, D., Machado, I.C., 2005. Pollination of *Campanula rapunculus* L. (Campanulaceae): How much pollen flows into pollination and into reproduction of oligolectic pollinators? Plant Syst. and Evol. 250(3), 147–156.

Snow, A.A., 1982 Pollination intensity and potential seed set in *Passiflora vitifolia*. Oecologia 55(2), 231–237.

Waites, A.R., Ågren, J., 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. J. Ecology 92(3), 512–526.

Waser, N.M., Price, M.V. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaeae): are ovules usurped. Am. J. Bot. 78(8), 1036-1043.

Young, H.J., Stanton, M.L. 1990. Influences of floral variation on pollen removal and seed production in wild radish. Ecology. 71(2), 536–547

Żebrowska, J. 1995. The viability and storage of strawberry pollen. Plant Breed. 114(5), 469-470.

Connecting text

Chapter 3 explores the efficiency of wild bees, managed bees, and species-rich bee assemblages as pollinators of strawberry, through the use of the pollen measurement techniques developed in Chapter 2. The research questions motivating this chapter are: 1) how do species richness and the frequency of flower visits affect the quantity of pollen deposited and removed from crop flowers and subsequent crop yield? 2) How do managed and wild pollinators perform as pollinators of strawberry, both individually and in tandem? The overarching goal of this chapter was to further knowledge on the comparative roles of species richness and community composition in the provision of ecosystem functions, using strawberry as a model system.

Author Contributions: The authors of this work are Gail MacInnis[1] and Jessica Forrest[2]. G.M. designed the study, collected the data, and conducted analyses with assistance from J.F. G.M. wrote the manuscript with input and edits from J.F.

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Chapter 3: Pollination by wild bees yields larger strawberries than pollination by honey bees

3.1 Abstract

A diverse array of wild bee species may provide more effective pollination than the widely employed European honey bee (*Apis mellifera* L.). High species richness within crop pollinator assemblages has been linked to enhanced fruit and seed yields, but species richness is often confounded with abundance in studies of pollinator communities. We investigated the effects of bee diversity and species identity on pollen deposition and crop yield in the strawberry (*Fragaria* x *ananassa*) variety *Jewel* through a field experiment that independently manipulated the species richness and abundance of flower visitors. We used a new pollen deposition measurement technique to determine the pollen contribution of individual bees in an assemblage of flower visits. We compared the performance of wild bee species and managed honey bees, as pollinators of strawberry. We also calculated the influence of species richness, visit frequency, and visitor identity on fruit mass, using the fruit that developed from each sampled flower. Species richness of flower visitors did not influence floral pollen loads or strawberry mass. Honey bees and wild bees deposited the same amount of pollen per visited flower. However, strawberries that developed from flowers visited by wild bees were heavier than flowers visited by honey bees. In addition, flowers visited by a combination of wild and honey bees produced strawberries that weighed less than flowers receiving purely wild bee visits. Our findings show that honey bee pollination results in lower yields than wild bee pollination in a strawberry crop. Consequently, if managed honey bees in strawberry fields displace wild pollinators, growers may obtain suboptimal yields. Management efforts aimed at the maintenance or enhancement of wild pollinator populations may therefore be a cost-effective way to increase both crop yield and biodiversity on strawberry farms.

3.2 Introduction

Species-rich pollinator communities have been associated with enhanced fruit and seed yields in agricultural settings (Klein et al. 2003; Vergara & Badano 2009; Brittain et al. 2013; Mallinger & Gratton 2015). Temporal and spatial complementarity among pollinators can allow pollen transfer to a greater number of plant species within communities (Fründ et al. 2014), plant individuals within populations (Hoehn et al. 2008; Martins et al. 2015), and even stigmas within flowers (Chagnon et al. 1993). Direct interference among pollinator species can also increase the pollination effectiveness of managed honey bees (Degrandi-Hoffman & Watkins 2000; Greenleaf & Kremen 2006; Brittain et al. 2013). However, some of the observed correlations between pollinator species richness and crop yield may simply reflect the fact that richness and abundance are typically confounded in natural settings (e.g., Klein et al. 2003; Hoehn et al. 2008; Rogers et al. 2014). The same yield benefits may be achieved by increasing abundance of a few species rather than increasing diversity (Winfree et al. 2015). To fully understand the relationship between crop yield and the composition of the pollinator community, information is needed on the pollen deposited by each flower visitor in an assemblage of visits, and its link to fruit yield.

Partitioning the effects of pollinator species richness, abundance, and identity on fruit yield is challenging. It is labour-intensive to determine individual species' pollen contributions by pollen-counting, so pollinator performance is often measured using the visit frequency of different pollinator guilds—a reasonable but coarse approach (Vázquez et al. 2005; Klein et al. 2012; Martins et al. 2015). Visit frequency measures are sometimes combined with measures of single-visit effectiveness (the number of pollen grains, fruit, or seeds resulting from a single pollinator visit to a flower) as a measure of pollinator performance (Greenleaf & Kremen 2006, Winfree et al. 2007; Rader et al. 2009; Garibaldi et al. 2013). This approach assumes that all visitors' contributions to fruit or seed set are additive and consistent within a species, which may not be the case. The relevance of single-visit deposition measurements to fruit-set under more natural (multiple-visit) conditions is questionable, as stigmas can become saturated with pollen during the first few visits, and successive visitors may fail to increase (and may even decrease) fruit set (Morris et al. 2010; Sáez et al. 2014). Furthermore, studies that measure pollen deposition by flower visitors generally do so in a laboratory, after a flower has been removed from the field (Wilmer et al. 1994; Artz & Nault 2011; Brittain et al. 2013), making it impossible to directly link pollen deposition to fruit or seed set, the variables that are of most interest to growers. These methodological challenges limit our ability to make informed recommendations on the pollinator assemblages most beneficial to growers.

In this study, we measure floral pollen loads in strawberry through stigmagraphs, a new pollen deposition measurement technique (MacInnis & Forrest 2017) that allows us to overcome many of the limitations of previous work on pollinator effectiveness. Stigmagraphs are high-resolution macro photographs taken of a flower's stigmas after it receives a visit from a pollinator. These photographs are processed though free, open-source image-analysis software (ImageJ-Fiji) to calculate the pollen contribution of each visitor in an assemblage of flower visitors. This method allows the sampled flowers to remain undisturbed in the field for direct fruit analyses. We used this novel approach to 1) explore the influence of pollinator species richness on pollen deposition and strawberry mass, while controlling for pollinator abundance, and 2) investigate the effects of pollinator identity (particularly honey bee vs. wild bee) and visit frequency on the quantity of pollen deposited and strawberry mass. We also examined pollen deposition and strawberry development on flowers receiving a combination of honey bee and wild bee visits, in light of previous evidence that complementarity in within-flower foraging behaviours of honey bees and wild bees can improve pollination in strawberry (Chagnon et al. 1993). Specifically, we expected that flowers receiving visits by abundant and species-rich bee assemblages would have larger pollen loads and thus higher fruit yields than those receiving fewer visits, or visits by fewer species.

3.3 Methods

3.3.1 Study system

The study was conducted on a commercial multi-crop farm in Île Perrot, Québec, Canada (45°21'57.7"N 73°55'35.6"W), in 2016. The farm contained 6 hectares of June-bearing strawberries, planted in May 2015. All sampling was done on the *Jewel* variety, which is commonly grown in the region and was the most abundant on the farm (10 rows of 80 m).

Other varieties grown within the 6 hectares included *Annapolis*, *Cavendish*, *Mira*, *Red Merlin*, *Sparkle, Honeoye*, and *Valley Sunset*.

We used only secondary flowers in inflorescences (150–200 stigmas per flower), as they are more abundant than primary flowers and are less prone to frost damage. The entire field site was drip-irrigated as needed, and fungicide/pesticide applications occurred on three occasions during the growing season. There were 32 managed honey bee hives on site. A preliminary survey in 2015 indicated that there was a species-rich wild bee community on the farm that consisted mostly of halictid and andrenid bees. Hereafter, ‘wild bee’ refers to any bee that was unmanaged on site (whether native or introduced).

We used strawberry mass as an indicator of pollination and strawberry quality. In Canada, strawberry diameter and shape are used to grade strawberries (The Canadian Food Inspection Agency, 2017). However, strawberry mass and diameter are highly correlated ($r_S = 0.90$; MacInnis, unpublished data) and most strawberries weighing less than 5.0 g do not meet the marketable strawberry grade requirement (16 mm diameter). To produce a fully-formed, marketable strawberry, the majority of ovules within a strawberry flower must be fertilized; unfertilized ovules do not stimulate growth of the receptacle and produce small or misshapen strawberries (Nitsch 1950). As such, insect pollination can increase yields, reduce malformations, and increase the shelf life and value of strawberries (Goodman & Oldroyd 1988, Klatt et al. 2014; Wietzke et al. 2018). Strawberry pollination is typically achieved by deposition of both autogamous (self) and allogamous (outcross) pollen through the action of wind, gravity, and insects (Darrow 1966; McGregor 1976). Cross-pollinated strawberry flowers often produce larger berries with fewer malformations than flowers receiving self (geitonogamous or autogamous) pollen (Pion et al. 1980; Chagnon et al. 1993); this effect is greater for certain inter-variety pollinations (Colbert & de Oliveira 1992). However, the degree of self-fertility differs among varieties (Żebrowska 1998) and the influence of outcrossed pollen on berry size would depend on the level of self-compatibility within a variety.

3.3.2 Data collection and experimental design

Data collection took place from 31 May to 17 June between 9h00 and 17h00. All data were collected during 7–8 hour periods in similar weather conditions: sunny days with little wind and temperatures of 19–30°C. This resulted in 16 days of data collection over the 18-day blooming period.

Three “control” treatments were applied to individual flowers (one per plant) on three occasions during bloom, and flowers in all treatments were labelled with a piece of adhesive tape around the stem. On each of the 2nd, 7th, and 12th days of sampling, 10 flowers were arbitrarily selected and assigned to treatments: bagged (pollinator-excluded), hand-pollination, and open pollination (n=30 flowers per treatment). Bagged flowers were covered with ‘No-see-um’ mesh (BioQuip, CA, USA) at the bud stage and left covered until petal abscission, signaling the end of receptivity. These flowers were assumed to be predominantly self-pollinated and used to evaluate strawberry development in the absence of pollinators. Hand-pollinated flowers were outcrossed by hand using three pollen donor flowers from at least three different non-*Jewel* rows. Each flower was hand-pollinated twice over two days to ensure all stigmas were receptive and received pollen. Open-pollinated flowers were unmanipulated; bees were allowed to freely visit throughout bloom. Comparing open-pollinated flowers to hand-pollinated flowers provides an estimate of pollen limitation.

For honey bee (HB) and wild bee (WB) treatments, plants with unopened secondary flowers were arbitrarily chosen and covered with mesh bags each day. Once in bloom, one flower per plant was chosen and assigned to a species richness or abundance treatment group (described below). The bag was removed when an observer was ready to watch for a visit. To accomplish the WB treatments, HBs were waved away as they approached the sample flower, and vice versa for the HB treatments. After each visit, the plant was carefully re-bagged to prevent additional pollen deposition. However, if a visitor hovered and then visited the focal flower again before bagging, this was counted as a second visit. This re-visiting behaviour was most often observed in honey bees; wild bees rarely visited the same flower twice in succession. Once a WB visitor had left the sample flower, it was net-collected and later identified to species following Asher and Pickering (2016). Some *Lasioglossum* could not be identified to species but were determined to belong to the *Lasioglossum viridatum* species group (Mitchell 1960). As all but one of our flower visitors were female bees, we considered only females in analyses. During bee visits, we also observed and recorded whether bees foraged for nectar, pollen, or both, as each of these foraging behaviours can influence pollination effectiveness (Thomson & Goodell 2001; Javorek et al. 2002; Monzón et al. 2004).

We used stigmagraphs (MacInnis & Forrest 2017) to determine each bee's contribution to the total pollen load on the sampled flowers. Specifically, after each bee visit, a high-resolution photograph of the flower's stigmas (stigmagraph) was taken and the amount of pollen on the stigmas was determined by differentiating the colour of the pollen from that of the stigmatic surface using the image-analysis software ImageJ-Fiji (Schindelin et al. 2012). The net pollen deposited (amount of pollen after the current visit minus the pollen present before the visit) and the cumulative pollen deposited (total pollen deposited over all visits) were then calculated from each stigmagraph. Here, pollen deposition was quantified as the number of pollen-bearing stigmas per bee visit per flower. Stigmagraphs of each sample flower were also taken before any bee visits to obtain an estimate of autogamous pollen deposition, as anthers frequently dehisced before stigma receptivity. Once a visit sequence was complete (typically within 10–50 minutes), the sampled flower was labelled and covered until the end of receptivity (petals wilted and stigmas browned). When ripe (red and easily separated from the pedicel), strawberries were taken directly to the laboratory and weighed immediately to avoid water loss.

3.3.3 Visitor species richness

Species richness of visitors was manipulated by selective exclusion as described above, on flowers from a group of arbitrarily selected plants (one flower per plant). The number of wild bee visits was kept constant at 4 per flower (based on Chagnon et al. 1989) to ensure full pollen deposition within flowers, and stigmagraphs were taken after each visit. Visitors could not be reliably identified to species in the field, so we controlled only morphospecies richness (1 to 4 morphospecies) during treatments. However, the majority of visitors were net-collected and later identified to taxonomic species, the predictor variable used in analyses. We attempted to obtain equal numbers of each richness level each day, but this was not possible owing to the seasonal increase in wild bee abundance ($r_s = 0.56$ between date and number of wild bee visits), and the inability to distinguish species in the field. Once each sampled flower developed into a strawberry, it was taken to the lab directly after harvest where its mass was recorded.

3.3.4 Visitor abundance and identity

Each day, one flower per plant was chosen and assigned to a visitor-abundance treatment (1, 2, 3, 4, or 6 bee visits) and a visitor-identity treatment (honey bee [“HB”] or wild bee [“WB”]). We attempted to obtain equal numbers of each treatment level each day, but the seasonal increase in WB abundance, noted above, made this impossible. As flowers were generally saturated with pollen after 3 visits, stigmagraphs were not taken after the 5th visit in a sequence. Flowers used for per-visit (net) pollen deposition analyses often received subsequent visits, so sample sizes differ between the net and cumulative pollen deposition visit groups.

During the species-richness and abundance experiments, honey bees entered many (14%; *n* = 49) of the flowers that we intended to be WB flowers. We included these flowers in *post-hoc* analyses of the effects of bee identity on strawberry mass and as an additional “Mixed” bee identity treatment.

3.3.5 Data analysis

We analysed three response variables: net pollen deposition, cumulative pollen deposition, and strawberry mass for HB and WB flowers. “Pollen deposition” refers to the number of pollen-bearing stigmas in each strawberry flower. Strawberries that developed from flowers that bloomed after the 12th day of sampling were considerably smaller than those that bloomed earlier ($r_s = -0.58$ between sampling day [1–16] and mass). Therefore, only flowers from the first 12 days of sampling were included in yield analyses.

All statistical analyses were conducted with R v.3.3.3 (R Core Team 2017). We used generalized linear models (GLMs) in the R package MASS (Venables & Ripley 2002) to (a) test the assumption that all flowers had similar amounts of autogamous pollen prior to visit sequences, (b) compare pollen deposition and strawberry mass among hand-supplemented, bagged, and open control flowers, (c) test the effects of species richness, bee identity, and abundance on pollen deposition and strawberry mass, (d) compare pollen deposition at each visit level to that of the hand-supplemented flowers, and (e) compare pollen deposition and strawberry mass of flowers receiving a mixture of wild and honey bee visits to that of flowers visited solely by wild or honey bees. For the species richness models, species richness (1–4 spp. per flower) was tested as a categorical predictor of total pollen per flower and strawberry mass. For the bee abundance models, we tested the effects of bee identity (HB, WB) and the number of bee visits (1–4, 6) as categorical predictors of the net and cumulative number of pollen-bearing stigmas, and strawberry mass. In analyses including Mixed-visited (HB + WB) flowers, we compared strawberry mass among bee groups (HB, WB, Mixed), then categorized all Mixed flowers according to the identity of the first visitor (HB or WB) and tested the influence of the first visitor on strawberry mass.

To investigate differences in pollen deposition or strawberry mass among wild bee genera, we combined the visitor-abundance, species-richness, and Mixed-visited flower datasets (n = 359 flowers) and classified flowers according to the genus that comprised the majority of visits to the flower. If one genus did not comprise over half the visits to a flower, it was classified as a 'Diverse' flower. We then used two GLMs to test the influence of bee genus on pollen deposition and strawberry mass.

For all GLMs (described above) with pollen deposition as a response variable, a negative binomial error distribution with a log link function was used to account for overdispersion in the pollen deposition data. To account for the variability in stigmas per flower, we included the number of stigmas as an offset in each pollen model. For the GLMs with strawberry mass as a response variable, a tweedie distribution with an inverse link function was implemented using R packages 'tweedie' (Dunn 2005) and 'statmod' (Giner & Smyth 2016). The tweedie distribution was used to account for the zero-inflation in the mass data, as many fruits failed to develop. Sampling date was included as a covariate in all strawberry mass models to account for the influence of plant age and temperature on strawberry plant growth and fruit development (Le Mière et al. 1998). All pairwise comparisons were conducted with the 'multcomp' package

(Hothorn et al. 2008) and where necessary the ‘sandwich’ package (Zeileis 2004) to compare means with non-normal, heteroscedastic errors (Herberich et al. 2010).

3.4 Results

In total, 663 bee visits were recorded to 359 flowers over the 12 collection days. Of these, 362 were honey bee (HB) visits and 301 were wild bee (WB) visits, made by 34 species in eight genera. The most abundant were species of *Lasioglossum* and *Andrena* (see Table S3.1 for full species list).

For the ‘control’ flowers, 22 hand-pollinated, 24 open-pollinated and 20 bagged flowers were successfully harvested for analyses. All flowers that received bee visits produced berries that were significantly heavier (HB: mean ± SD, 7.47 ± 6.32 g, WB: 12.77 ± 8.20 g, $\chi^2_2 = 63.16$, $p < 0.001$) than berries produced by the bagged (pollinator-excluded) flowers (3.34 ± 4.07 g), suggesting that insect visits are essential for the production of marketable *Jewel* strawberries. Strawberries from open-pollinated flowers did not differ significantly in mass (9.40 ± 9.39 g, $\chi^2_1 = 0.004$, $p = 0.95$) from hand-pollinated flowers (11.71 ± 5.97 g), which suggests that plants were not pollen-limited.

3.4.1 Visitor species richness

In total, 98 flowers receiving 4 WB visits each (392 visits) were used for the species richness analyses. Total pollen deposition did not vary with species richness of flower visitors ($\chi^2_3 = 2.98$, $p = 0.39$, Fig. 3.1A; Table S3.2), nor did strawberry mass ($\chi^2_3 = 3.06$, $p = 0.38$; Fig. 3.1B; Table S3.2).

3.4.2 Visitor abundance and identity

Net pollen deposition

In total, 210 HB visits and 169 WB visits were included in the analysis of net pollen deposition (due to the speed of some bee visits, stigmagraphs were not captured for all flower visits). Net pollen deposition varied with visit number ($\chi^2_4 = 49.02$, $p < 0.001$) but not with bee identity ($\chi^2_1 = 1.34$, $p = 0.25$; Fig. 3.2); nor was there a significant interaction between bee identity and visit number ($\chi^2_4 = 3.94$, $p = 0.41$). The net pollen deposited per visit decreased as stigmas became saturated with pollen: fewer than eight additional stigmas per flower received pollen by the sixth visit during either HB or WB visit sequences (Fig. 3.2, Table S3.1).

Cumulative pollen deposition

Some strawberries were lost to accidental harvesting by farmers; only pollen from those flowers in which fruit measurements were taken were used in cumulative (total) pollen analyses. In total, 115 HB flowers and 97 WB flowers were measured for cumulative pollen deposition ($n = 212$). The amount of self-pollen deposited prior to insect visitation did not vary with number of visits per flower (mean ± SD, 98.26 ± 50.37, $\chi^2{}_4 = 2.52$, $p = 0.64$) or bee type ($\chi^2{}_1 = 0.13$, $p = 0.72$, Table S3.3). Total pollen deposited per flower varied with visit number ($\chi^2{}_4 = 13.78$, $p = 0.008$), but not with bee identity ($\chi^2{}_1 = 0.97$, $p = 0.32$), nor did the effect of visit number depend on bee identity (bee identity × visit number interaction: $\chi^2{}_4 = 0.29$, $p = 0.99$, Fig. 3.3; Table S3.3). The total pollen deposited in flowers visited by two bees was significantly more than flowers visited by one bee ($z = 3.63$, $p = 0.001$), but beyond two visits, the total number of pollen-bearing stigmas did not differ significantly from the hand-pollinated controls, regardless of bee type (visits 3–6, $p > 0.05$; Fig. 3.3; Table S3.3). This suggests that stigmas were saturated with pollen at three bee visits.

The total pollen deposited in flowers visited by a mixture of honey and wild bees (Mixed) did not differ from that of flowers visited solely by WBs or HBs ($\chi^2{}_2 = 0.18$, $p = 0.91$).

Strawberry yield

WB flowers produced strawberries that were heavier than HB berries at all visit levels except four visits, at which the difference was marginal (Table S3.4, Fig. 3.4D). Including data from all visits, strawberry mass was influenced by bee identity ($\chi^2{}_1 = 42.27$, $p < 0.001$) but not by number of visits ($\chi^2{}_4 = 0.45$, $p = 0.97$). There was no interaction between bee identity and visit number ($\chi^2{}_4 = 1.95$, $p = 0.74$). Overall, HB-visited flowers produced strawberries that weighed 42% less (mean ± SD, 7.47 ± 6.32 g) than WB-visited flowers (12.77 ± 8.20 g, $z = 5.81$, $p < 0.001$, Fig. 3.4F).

Strawberries produced from Mixed flowers weighed 34% less (8.38 ± 6.21 g, $z = 4.69$, $p < 0.001$) than WB-visited flowers (12.77 ± 8.20 g), but did not differ in mass from strawberries produced from HB-visited flowers (7.47 ± 6.32 g, $z = 0.93$, $p = 0.61$, Fig. 3.5A). Furthermore, after categorizing flowers by their first visitor, Mixed flowers that received a visit by a HB first were 48% lighter (6.68 ± 5.75 g, $z = 2.24$, $p = 0.025$) than those that received a WB visit first (12.77 ± 8.19 g, Fig. 3.5B).

Pollination by genus

Strawberry mass varied with visitor identity (bee genus; $\chi^2_6 = 104.91$, $p < 0.001$; Fig S3.1B), but total pollen deposited did not ($\chi^2_6 = 3.04$, $p = 0.80$; Fig S3.1A). Strawberry mass was highest for flowers that received most of their visits from bees in the genera *Lasioglossum* (mean ± SD, 16.68 ± 8.46 g) and *Augochlorella* (16.22 ± 5.64 g). Strawberry mass was lowest for flowers that received most of their visits from honey bees (7.47 ± 6.32 g, Fig S3.1B).

3.5 Discussion

Species richness is often confounded with bee abundance in studies that use natural diversity gradients to explore the relationship between pollinator diversity and crop yield (Klein et al. 2003; Hoehn et al. 2008; Mallinger & Gratton 2015). Attempts to disentangle the influence of abundance and species richness on fruiting have involved simple bee and plant communities assembled in mesocosms (Fontaine et al. 2006; Fründ et al 2013) or sophisticated analytical approaches based on single-visit pollen deposition measurements (Winfree et al. 2015; Genung et al. 2017). To our knowledge, our study presents the first experiment to separate the contributions of individual flower visitors to pollination at the community level in the field. In decoupling the influence of bee abundance and richness, we found that bee identity had the greatest influence on strawberry yield. On average, wild bee-visited flowers yielded strawberries that weighed more than strawberries produced by honey bee-visited flowers. Although we only allowed a finite number of visits to each flower, this pattern would likely have held regardless of visit number, as stigmas were fully saturated at three visits (Fig. 3.3). As so few bee visits were required to saturate stigmas, the potential for species richness to affect pollination and yield was limited.

Overall, the wild bee species in this system were the most effective pollinators of strawberry. Similar results were found in another recent comparison of strawberry pollinators (Horth &

Campbell 2018). Here, *Lasioglossum* and *Augochlorella* bees yielded the largest strawberries (Fig. S3.1), suggesting that bees in these genera are highly effective pollinators of strawberry. We observed that these small bees (5–7 mm in length) were able to forage for both pollen and nectar without shifting the anthers, whereas larger bees (>10 mm) often bent and moved anthers toward the stigmas while searching for nectar. The latter behaviour may have increased autogamous pollen deposition, which can potentially reduce fruit or seed set relative to allogamous pollen (Waser & Price 1991). We also observed that the majority of wild bees in this community actively collected pollen in most of their flower visits, with the exception of *Halictus* bees. This may have facilitated relatively more pollen transport between plants than was achieved by nectar-seeking visitors (honey and *Halictus* bees). Indeed, flowers pollinated by honey bees and *Halictus* bees produced strawberries of similar mass (Fig. S3.1).

The greater mass of wild bee-pollinated berries suggests that the wild bee community at our study site delivered more outcrossed pollen than managed honey bees, as both bee groups deposited similar amounts of pollen per visit (Fig. 3.2). The influence of pollen quality (allogamous versus geitonogamous or autogamous) is a seldom-quantified (but see Alonso et al. 2012) but important consideration when predicting pollinator influence on crop yield (Aizen and Harder 2007). Further, in strawberry, certain inter-variety crosses have been found to increase berry mass (Colbert & de Oliveira 1992). As our study was done on one commercial berry farm over one season, yield may have been influenced by the strawberry varieties specific to the study site. In general, the potential of insect pollination to increase yield in modern strawberry fields will depend on the pollinator community composition, resource availability, the degree of self-compatibility within varieties, the mix of strawberry varieties planted and possibly their spatial configuration. As commercial strawberry plants are usually propagated by cloning, and strawberries of the same variety are often planted in adjacent rows, pollinators that tend to forage within-rows may reduce the potential for cross-pollination. In several cropping systems, honey bees have been found to forage consecutively on plants in the same row (Free 1962; Greenleaf & Kremen 2006; Brittain et al. 2013), and we have observed a similar foraging pattern in strawberry. We are currently conducting a separate study to directly investigate the influence of pollinator foraging patterns on strawberry yield.

Finally, our results showed that pollen deposition was not a linear function of visit frequency, but declined exponentially (Fig. 3.2). Measuring pollination as a product of single-visit deposition and visit frequency (e.g., Kremen et al. 2002; Klein et al. 2003; Winfree et al. 2007; Brittain et al. 2013; Garibaldi et al. 2013; Rogers et al. 2014; Pfister et al. 2017) may provide a reasonable estimate of a flower visitor's pollen-deposition potential, but it is difficult to know whether that potential will be realized in a community setting, where species interactions become important. In real-world systems, the dominant pollinator species may have more influence on pollen deposition and fruit and seed yields (Winfree et al. 2015, Genung et al. 2017). This has important implications for cropping systems: if a less-effective managed pollinator is supplied in abundance, it may usurp ovules that could have been fertilized by more effective pollinators, and prevent potential yield gains. Indeed, in our system, flowers that were first visited by a honey bee yielded lighter strawberries than did flowers that received a wild bee visitor first (Fig. 3.5B).

3.5.1 Implications

Although honey bees generally produce marketable strawberries (Chagnon et al. 1989; Albano et al. 2009; this study), strawberries pollinated by honey bees in our study weighed on average 42% less than wild bee-visited flowers. Not only is it costly for growers to rely on managed honey bees for pollination, but the lower weight of honey bee-pollinated strawberries may result in additional financial losses. Managed honey bees may compete with more-effective wild pollinators, saturate strawberry stigmas with lower quality pollen, and result in suboptimal yields. However, the potential for wild bees to provide full pollination at the crop level would depend on their abundance. As most of the wild bee species in our study were ground-nesters, dedicating a proportion of cultivated land to untilled earth, preserving semi-natural habitat, reducing competition with honey bees, and providing floral resources outside of crop blooming periods may increase their abundance on-farm and directly benefit crop yield (see also Isaacs et al. 2009; Garibaldi et al. 2014). If crop yields can be maintained or increased over a smaller area through higher-quality pollination, reducing cultivated land may become a more attractive option for farmers, while simultaneously enhancing wild pollinator populations and biodiversity on farms.

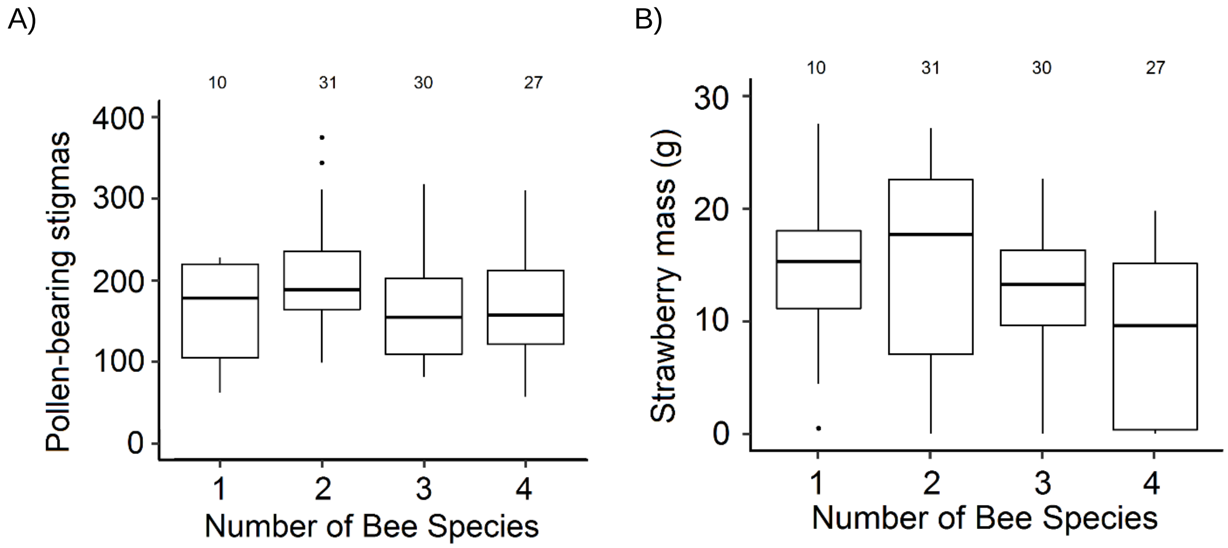

Figure 3.1. Total number of pollen-bearing stigmas (A) and strawberry mass (B), as functions of visitor species richness. Flowers received four bee visits in each treatment group. Boxes show the interquartile range, the median is indicated by a horizontal line, whiskers indicate the data range, and points are outliers. Sample sizes are listed above. Pollen deposition and strawberry mass did not vary significantly with species richness of flower visitors.

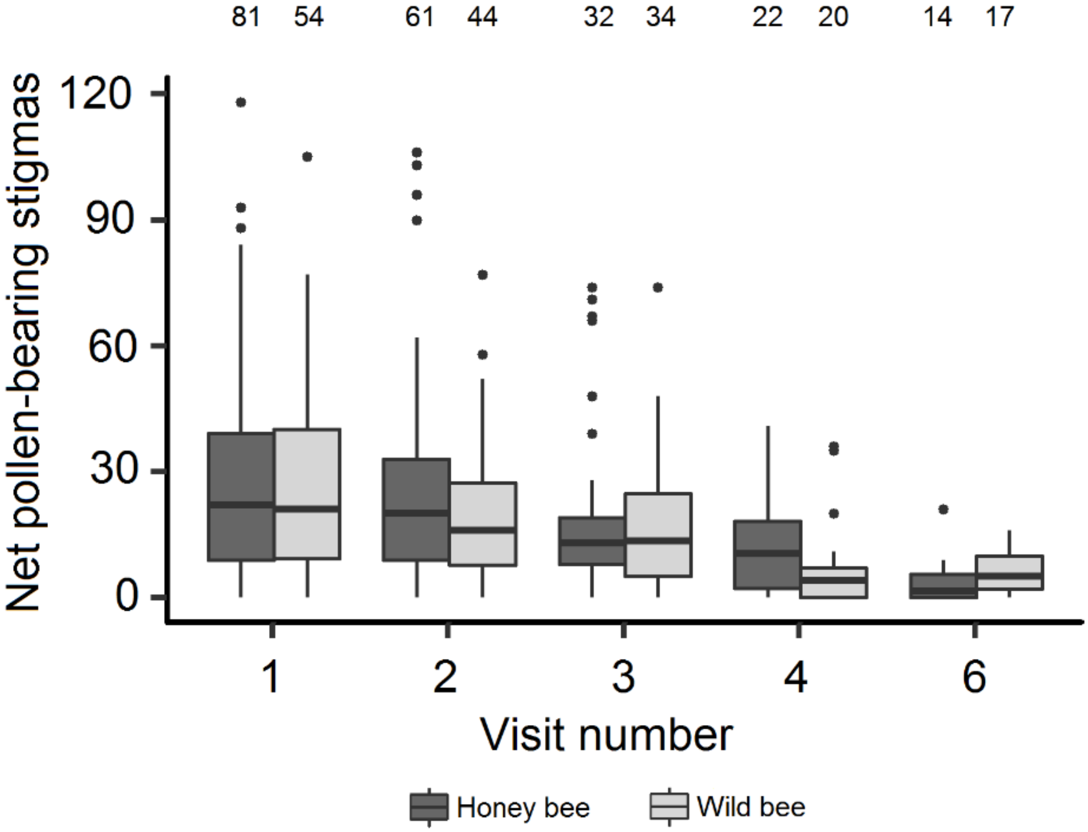

Figure 3.2. Net pollen-bearing stigmas for a given number of visits by honey bees and wild bees to strawberry flowers. Sample sizes are listed above each box. There were no significant differences between wild bees and honey bees in the net pollen deposited at any visit number.

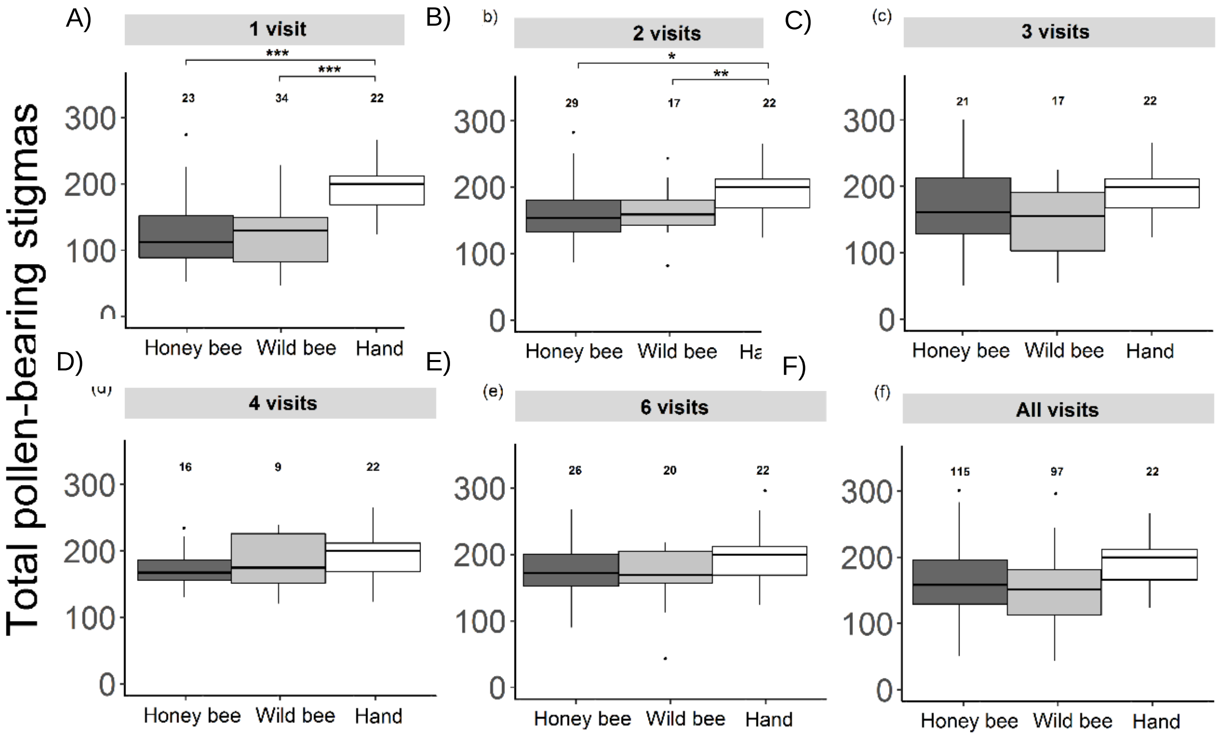

Figure 3.3. Total pollen-bearing stigmas for strawberry flowers receiving 1–6 visits (A–E), and over all visit numbers combined (F), by different visitor types. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Sample sizes are listed above. The hand-pollinated control is shown in each panel to facilitate comparison.

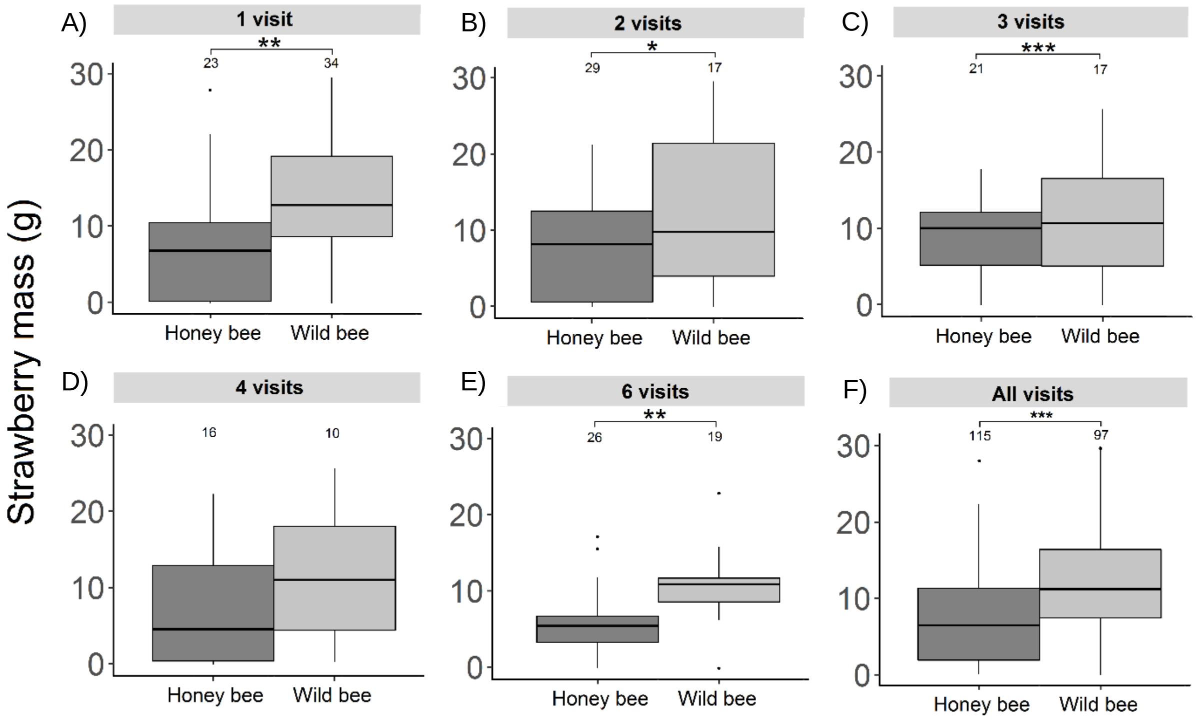

Figure 3.4. Strawberry mass of flowers receiving 1–6 visits (A–E), and over all visits (F) by different visitor types. Sampling date was included as a covariate in all comparisons of strawberry mass. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Sample sizes are listed above.

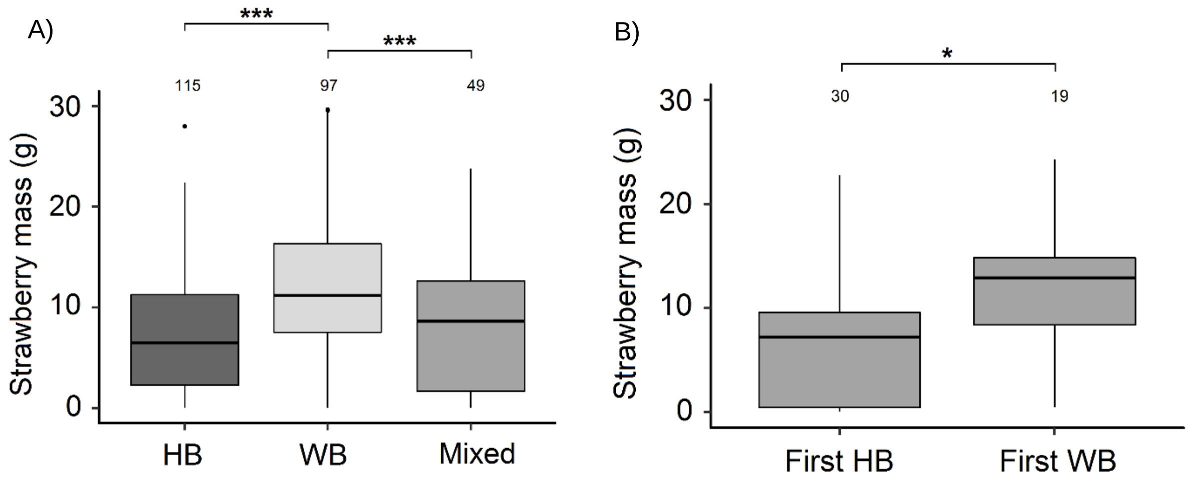

Figure 3.5. The impact of bee identity (A) and the identity of the first visitor (B) on yield in strawberry flowers receiving multiple bee visits. “Mixed” strawberries are those developed from flowers receiving visits by a combination of honey bees and wild bees during the abundance and species richness experiments. “First HB” strawberries were produced from Mixed flowers that had a honey bee visit first. “First WB” strawberries were produced from Mixed flowers that had a wild bee visit first. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Sample sizes are listed above.

3.6 Acknowledgements

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3.7 References

Aizen, M.A., Harder, L.D., 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology. 88(2), 271-281.

Albano, S., Salvado, E., Duarte, S., Mexia, A., Borges, P. A., 2009. Pollination effectiveness of different strawberry floral visitors in Ribatejo, Portugal: selection of potential pollinators. Part 2. Adv. Hortic. 23(4), 246-253.

Alonso, C., Herrera, C.M., Ashman, T.L., 2012. A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. New Phytol. 193(2), 532-542.

Artz, D.R., Nault, B.A., 2011. Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as pollinators of pumpkin. J. Econ. Entomol. 104(4), 1153-1161.

Ascher, J.S. Pickering, J., 2016. Discover life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Retrieved from http://www.discoverlife.org/mp/20q?guide=Apoidea_species

Brittain, C., Williams, N., Kremen, C. Klein, A., 2013. Synergistic effects of non-*Apis* bees and honey bees for pollination services. Proc. R. Soc. Lond., B, Biol. Sci., 280(1754), 20122767.

Chagnon, M., Gingras, J., De Oliveira, D., 1989. Effect of honey bee (Hymenoptera: Apidae) visits on the pollination rate of strawberries. J. Econ. Entomol. 82(5), 1350-1353.

Chagnon, M., Gingras, J. de Oliveira, D., 1993. Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). J. Econ. Entomol. 86(2), 416–420.

Colbert, S., de Oliveira, D., 1992. Pollinisation croisée et production de quatre cultivars de fraisier, *Fragaria* × *ananassa*. Can. J. Plant Sci. 72(3), 857-861.

Darrow, G. M., 1966. The strawberry. New York, NY: Holt, Rinehart and Winston.

DeGrandi-Hoffman, G., Watkins, J. C., 2000. The foraging activity of honey bees *Apis mellifera* and non-*Apis* bees on hybrid sunflowers (*Helianthus annuus*) and its influence on cross-pollination and seed set. J. Apicult. Res. 39(1-2), 37-45.

Dunn, P. K., Smyth, G. K., 2005. Series evaluation of Tweedie exponential dispersion models. Statistics and Computing, 15(4), 267-280.

Fontaine, C., Dajoz, I., Meriguet, J. Loreau, M., 2006. Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. PLoS bio. 4(1), e1.

Free, J.B., 1962. The effect of distance from pollinizer varieties on the fruit set on trees in plum and apple orchards. J. Hortic. Sci. 37(4), 262–271.

Fründ, J., Dormann, C.F., Holzschuh, A. Tscharntke, T., 2013. Bee diversity effects on pollination depend on functional complementarity and niche shifts. Ecology 94(9), 2042–2054.

Garibaldi, L.A., Carvalheiro, L.G., Leonhardt, S.D., Aizen, M.A., Blaauw, B.R., Isaacs, R., Kuhlmann, M., […], Winfree, R., 2014. From research to action: enhancing crop yield through wild pollinators. Front. Ecol. Environ. 12(8), 439–447.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., […], Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127), 1608–1611.

Genung, M.A., Fox, J., Williams, N.M., Kremen, C., Ascher, J., Gibbs, J., Winfree, R., 2017. The relative importance of pollinator abundance and species richness for the temporal variance of pollination services. Ecology 98(7). 1807-1816.

Giner, G., Smyth, G.K., 2016. statmod: probability calculations for the inverse Gaussian distribution. R Journal, 8, 339-351.

Goodman, R., Oldroyd, B., 1988. Honeybee pollination of strawberries (*Fragaria* x *ananassa* Duchesne). Aust. J. Exp. Agr. 28(3), 435.

Greenleaf, S.S., Kremen, C., 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. U.S.A. 103(37), 13890–13895.

Herberich, E., Sikorski, J., Hothorn, T., 2010. A robust procedure for comparing multiple means under heteroscedasticity in unbalanced designs. PLoS one 5(3), 1–8.

Hoehn, P., Tscharntke, T., Tylianakis, J.M. Steffan-Dewenter, I., 2008. Functional group diversity of bee pollinators increases crop yield. Proc. R. Soc. Lond., B, Biol. Sci. 275(1648), 2283–2291.

Horth, L. Campbell, L.A., 2018. Supplementing small farms with native mason bees increases strawberry size and growth rate. J. Appl. Ecol. 55(2), 591-599.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J. 50(3), 346-363.

Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., Landis, D., 2009. Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. Front. Ecol. Environ. 7(4), 196-203.

Javorek, S.K., Mackenzie, K. E., Vander Kloet, S.P., 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Ann. Entomol. Soc. Am. 95(3), 345-351.

Klatt, B.K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., Tscharntke, T., 2014. Bee pollination improves crop quality, shelf life and commercial value. Proc. R. Soc. Lond., B, Biol. Sci. 281(1775), 20132440.

Klein, A.-M., Steffan-Dewenter, I. Tscharntke, T., 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proc. R. Soc. Lond., B, Biol. Sci. 270(1518), 955–961.

Klein, A.-M., Brittain, C., Hendrix, S.D., Thorp, R., Williams, N. Kremen, C., 2012. Wild pollination services to California almond rely on semi-natural habitat. J. Appl. Ecol. 49(3), 723-732.

Kremen, C., Williams, N. M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. Proc. Natl. Acad. Sci. U.S.A., 99(26), 16812-16816.

Le Mière, P., Hadley, P., Darby, J., Battey, N.H., 1998. The effect of thermal environment, planting date and crown size on growth, development and yield of *Fragaria* × *ananassa* Duch. cv. Elsanta. J. Hortic. Sci. & Biotech. 73(6), 786-795.

MacInnis, G., Forrest, J.R.K., 2017. Quantifying pollen deposition with macro photography and 'stigmagraphs'. J. Pollinat. Ecol. 20(5), 13-21.

Mallinger, R.E. Gratton, C., 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. J. Appl. Ecol. 52(2), 323–330.

Martins, K.T., Gonzalez, A., Lechowicz, M.J., 2015. Pollination services are mediated by bee functional diversity and landscape context. Agric., Ecosyst. Environ. 200, 12-20

McGregor, S.E., 1976. Insect pollination of cultivated crop plants. US Department of Agriculture, Washington, DC

Michener, C. D., 2000. The bees of the world (Vol. 1). JHU press.

Monzón, V.H., Bosch, J., Retana, J., 2004. Foraging behavior and pollinating effectiveness of *Osmia cornuta* (Hymenoptera: Megachilidae) and *Apis mellifera* (Hymenoptera: Apidae) on “Comice” pear. Apidologie 35(6), 575-585.

Morris, W.F., Vázquez, D.P., Chacoff, N.P., 2010. Benefit and cost curves for typical pollination mutualisms. Ecology 91(5), 1276-1285.

Nitsch, J.P., 1950. Growth and morphogenesis of the strawberry as related to auxin. American J. Bot. 37, 211.

Pfister, S.C., Eckerter, P.W., Schirmel, J., Cresswell, J.E. Entling, M.H., 2017. Sensitivity of commercial pumpkin yield to potential decline among different groups of pollinating bees. Royal Soc. Open Sci. 4(5), 170102.

Pion, S., Oliveira, D.D., Paradis, R. O., 1980. Agents pollinisateurs et productivité du fraisier 'Redcoat', *Fragaria* x *ananassa* Duch. Phytoprotection 61, 72–78.

R Core Team, 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL:http://www.Rproject.org/.

Rader, R., Howlett, B.G., Cunningham, S.A., Westcott, D.A., Newstrom-Lloyd, L.E., Walker, M.K., Teulon, D.A.J. Edwards, W., 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. J. Appl. Ecol. 46(5), 1080–1087.

Rogers, S.R., Tarpy, D.R., Burrack, H. J. 2014. Bee species diversity enhances productivity and stability in a perennial crop. PLoS one 9(5), e97307.

Sáez, A., Morales, C.L., Ramos, L. Y., Aizen, M.A., 2014. Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. J. Appl. Ecol. 51(6), 1603-1612.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., […], Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Method. 9(7), 676-682.

The Canadian Food Inspection Agency 2017. The Canadian Grade Compendium, Volume 2: Fresh Fruit or Vegetables. Ottawa, ON, Canada.

Thomson, J.D. Goodell, K., 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. J. Appl. Ecol. 38(5), 1032–1044.

Vázquez, D.P., Morris, W.F., Jordano, P., 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. Ecol. Lett. 8(10), 1088-1094.

Venables, W.N. Ripley, B.D., 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Vergara, C.H., Badano, E.I., 2009. Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. Agric., Ecosyst. Environ. 129(1-3), 117–123.

Waser, N.M., Price, M.V., 1991. Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. Ecology, 72(1), 171-179.

Wietzke, A., Westphal, C., Gras, P., Kraft, M., Pfohl, K., Karlovsky, P., [...], Smit, I., 2018. Insect pollination as a key factor for strawberry physiology and marketable fruit quality. Agric., Ecosyst. Environ. 258, 197-204.

Willmer, P.G., Bataw, A.A.M., Hughes, J.P., 1994. The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. J. Econ. Entomol. 19(3), 271-284.

Winfree, R., Williams, N.M., Dushoff, J. Kremen, C., 2007. Native bees provide insurance against ongoing honey bee losses. Ecol. Lett. 10(11), 1105–1113.

Winfree, R., Fox, J.W., Williams, N.M., Reilly, J.R., Cariveau, D.P., 2015. Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. Ecol. Lett. 18(7), 626-635.

Zebrowska, J., 1998. Influence of pollination modes on yield components in strawberry (*Fragaria* x *ananassa* Duch.). Plant Breed. 117(3), 255–260.

Zeileis, A., 2004. Econometric Computing with HC and HAC Covariance Matrix Estimators. J. Stat. Softw. 11, 1-17.

Connecting text

The investigation of species richness and bee community composition in Chapter 3 revealed that community composition, specifically the frequency of wild bee visits, had a greater influence on pollination than did species richness. Wild bees, especially small-bodied bees in the genus *Lasioglossum*, were more efficient pollinators of strawberry than were honey bees. Chapter 4 investigates foraging behaviour as a potential mechanism behind the higher-quality pollination provided by wild bees. Through controlled experiments on a research farm, I assess the influence of field design and bee foraging behaviour (honey bee and wild bee) on the transfer of outcross pollen between strawberry plants. This chapter addresses the question: Can bee foraging behaviour interact with field design to affect the quality of crop pollination?

Author contributions: The authors of this work are Gail MacInnis[1] and Jessica Forrest[2]. GM conceived the ideas and designed the study, collected the data, conducted analyses, and wrote the manuscript. JF had input on experimental design, and provided critical contributions to the manuscript drafts.

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Chapter 4: Field design can affect cross-pollination and crop yield in strawberry (*Fragaria* x *ananassa* D.)

4.1 Abstract

The distance travelled by pollinators between successive flower visits can affect the quality of pollen transferred among plants. In cropping environments, especially monoculture systems, pollinators that travel between plants or rows may increase cross-pollination and consequently crop yield. However, the most commonly utilized crop pollinator, *Apis mellifera* L., tends to forage consecutively on nearest-neighbouring plants within rows. The level of cross-pollination can be further restricted in crops that are propagated by cloning. When a clonal variety is planted over large areas, the potential for outcrossed pollen deposition could be limited, regardless of pollinator flight distances. To investigate how pollinator movement and varietal diversity interact to affect crop pollination, we conducted an experiment with wild and honey bees in single- and multiple-variety strawberry fields. We hypothesized that the amount of cross-pollination provided by wild bees in multiple-variety strawberry fields would be greater than in single-variety fields, and greater than that provided by honey bees in either field type. We found that, indeed, flowers visited by wild bees produced larger strawberries than those visited by honey bees in multiple-variety plots, but only in the more self-incompatible of the two strawberry varieties tested. Strawberries resulting from honey bee pollination were of similar size regardless of the number of varieties planted in the field. Our results show that certain multiple-variety strawberry fields can benefit from the irregular foraging patterns of some solitary bee species, leading to increased cross-pollination and crop yield. Strawberry growers could take advantage of this effect by planting multiple varieties in close proximity and by supporting wild bee populations on farms.

4.2 Introduction

In pursuit of pollen and nectar, flower visitors dictate both the quantity and quality of pollen transferred between animal-pollinated plants (Lloyd & Schoen 1992; Harder and Barrett 1996). The pollen-transfer efficiency of crop pollinators is an important consideration for agricultural production as animal pollination is needed to maximize fruit and seed set for 70% of our main food crops (Klein et al. 2007). Of these crops, approximately 10% depend fully on animals to deposit outcrossed (or allogamous) pollen from other plants to set fruit, while the remainder have mixed-mating systems and can reproduce by both cross- and self-pollination. However, cross-pollination generally increases offspring fitness in mixed-mating systems (Darwin 1876; Charlesworth & Charlesworth 1987), and insect-pollination has been found to increase fruit yield or quality over self-pollination in crops such as cranberry (Sarracino & Vorsa 1991; Cane et al. 1996), strawberry (Klatt et al. 2014; Weitzke et al. 2018), oilseed rape (Bommarco et al. 2012), tomato (Morandin et al. 2001; de Melo e Silva Neto et al. 2013), and hot and sweet peppers (Raw 2000; Serrano & Guerra-Sanz 2006). In self-compatible plants, outcrossed fruits tend to have more—and more vigorous—seeds, which can have a competitive advantage in obtaining maternal resources over inbred seeds (reviewed by Stephenson 1981). Metaxenia is also a proposed mechanism driving enhanced fruit production in some self-compatible plants. Metaxenia refers to the influence of foreign pollen (typically from another variety) on the characteristics of the plant maternal tissues (Swingle 1928). Xenic effects have been found in crosses between certain varieties of apple, blueberry, raspberry (reviewed by Denney 1992) and strawberry (de Oliveira et al. 1983).

The probability that a pollinator transfers outcrossed pollen between plants depends on a complex set of variables, including the size and configuration of the plant population, and the foraging behaviours of pollinators (Levin & Kerster 1969; Levin 1981; Handel 1983; reviewed by Barrett 2003). High densities of flowering plants can increase pollinator abundance and flower visitation frequency, leading to reduced autogamous (within-flower) selfing (Schmitt 1983; Karron et al. 1995; Routley et al. 1999). Further, pollinators that visit few flowers per plant and/or move large distances between individual plants will tend to promote outcrossing and reduce geitonogamous (within-plant, among-flower) selfing (Lloyd & Schoen 1992; Cresswell et al. 2002; Ivey et al. 2003; Karron et al. 2009). However, cropping environments can add another degree of complexity to the relationship between pollinators and cross-pollination: although the high flowering plant densities in croplands may increase pollinator visitation frequency, the genetic diversity within certain crops can be low, particularly in monocrop fields populated by clones (e.g. blueberry, strawberry). In such fields, the potential for geitonogamous pollen transfer would be high, regardless of pollinator foraging patterns.

Studies linking pollen movement with bee foraging behaviours have mainly focused on natural plant communities (Waser 1982; Herrera 1987; Karron et al. 2009; Cranmer et al. 2012) and large-bodied bee species (e.g., *Bombus* spp.*,* Pyke 1978; Heinrich 1979; Zimmerman 1982; Thomson & Thomson 1989; Pyke & Cartar 1992; Karron et al. 1995; Goulson 2000; Javorek et al. 2002), including honey bees (Free 1962, 1968; Waddington 1980; Ginsberg 1986; Morris 1993; Walters & Schultheis 2009). These studies have found that large-bodied bees often exhibit a high degree of directionality, and generally arrive and depart a flower in the same direction. This tendency to fly straight ahead to the nearest neighbouring flower likely maximizes foraging efficiency, and/or reduces the probability of revisiting flowers (Pyke 1978). However, it is unclear whether foraging patterns observed in these large bee species are also typical of smaller bee species, which can often be the dominant pollinators on farms (Winfree et al. 2007; Mandelik et al. 2012; Forrest et al. 2015; Blitzer et al. 2016). The number of studies focused on the foraging patterns of small-bodied species is limited (Waddington 1979; Herrera 1987; Raw 2000), mostly likely due to the difficulty of tracking small bees. One study that successfully followed several small-bodied *Lasioglossum* bees found that although they typically exhibited a high degree of directionality, they also made occasional ‘looping’ flights, where the distance between visited flowers increased with each successive loop (Waddington 1979). This behaviour has not been observed in honey bees, the most widely employed crop pollinator.

The interactive effect of field design and pollinator movement on pollen transfer and fruit production within crops has been largely unexplored (but see Vezvaei & Jackson 1997; Raw 2000; Walters & Schultheis 2009). Here, we investigated the effects of field design and pollinator foraging patterns on fruit mass in strawberry in experimental fields dominated by managed honey bees and small, wild pollinators (*Lasioglossum* spp.). Wild bee pollination has been found to increase strawberry size over honey bee pollination (Horth & Campbell 2018; MacInnis & Forrest 2019), as has cross-pollination between certain strawberry varieties (Colbert & de Oliveira 1992; Tuohimetsä et al. 2014). The potential for strawberry yield to be increased through cross-pollination in the field depends on the propensity of foraging bees to successfully transfer allogamous pollen between plants, and on the genetic diversity within a crop. However, many commercial strawberry (*Fargaria x ananassa* Duch.) cultivars are vegetatively propagated through cloning in nurseries to preserve varietal traits. The cloned plants are subsequently sold and typically planted as field crops with one variety per row, for several rows or hectares consecutively. Pollinator foraging patterns may have a greater influence on outcrossing rates and subsequent crop yield in strawberry fields that contain multiple varieties (i.e. that are more genetically diverse) than in single-variety fields. To test whether wild bees transfer better-quality (outcrossed) pollen by moving more often between rows than honey bees, we controlled the visits of both bee types to strawberry flowers in fields with one variety and those with two varieties in adjacent rows, and measured subsequent fruit mass. We hypothesized that wild bee visitation would yield larger strawberries than visitation by honey bees, but only in multiple-variety fields, because of more frequent between-variety movements by wild bees.

4.3 Methods

4.3.1 Study system and experimental design

The study was conducted at The McGill Horticultural Research Centre in Ste-Anne-de-Bellevue, Québec, Canada (45°24’36.9”N 73°56’7.3”W), from May to October 2017. The research centre is situated in an urban–suburban landscape, with patches of agricultural and forested areas. Two popular commercial day-neutral strawberry varieties adapted to regional conditions were used for the study: *Seascape* (SS) and *Albion* (ALB). These two varieties were chosen as they had similar growth forms (plant height, leaf size), floral morphologies (corolla diameters and petal numbers), flowering densities, and bloom times (Bringhurst and Voth 1991; Shaw and Larson, 2006). Day-neutral strawberry varieties were used to increase the length of the sampling period, as they bloom consistently for several months. Bare-root strawberry plants were planted in May and bloom began in mid-July and continued until early October. The field plots were surrounded on all sides by several other crops, which provided alternative floral resources for pollinators periodically over the extended strawberry bloom. These crops included bell pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), zucchini (*Cucurbita pepo* L.), and asparagus (*Asparagus officinalis* L.).

A split-plot, 2 × 2 × 2 factorial design was used to test the influence of bee type (wild and honey bee), variety (SS, ALB) and field design (single-variety, multiple variety) on strawberry mass in experimental strawberry fields. We used a complete randomized block design with four blocks containing plots of each of the varieties and field types: single-variety SS, single-variety ALB and a multiple-variety plot of ALB and SS (MULT). The two bee treatments, wild bee (WB) and honey bee (HB), were assigned to individual plants within each plot each sampling day, as described below. Each of the twelve plots contained 4 rows of strawberry plants, 20 m in length (Fig. 4.1). The cultivation and spacing of plants corresponded to standard day-neutral cultivation practices. Strawberries were grown on raised beds covered with black plastic mulch (polyethylene), under which drip irrigation lines were installed. Plants were irrigated daily at a rate of 5 L/m^2. Soil moisture levels were monitored with a tensiometer and the irrigation schedule was adjusted as needed. The spacing was 0.2 m between plants within rows and 1.2 m between rows (OMAFRA, 2016).

The climate and overall growing conditions were average for strawberry in the study region (ISQ, 2017). Precipitation was above average in 2017 but sampling was only done on warm, sunny days (air temperature > 20 ° C) with little wind (<10 km/h). Sampling was done between 9h and 17h and all sampled plants were in the two middle rows of each plot, at least 1 m from the edge in any direction to avoid edge effects. As day-neutral strawberries have several blooming periods, some plants received a second treatment at subsequent blooms. Previous work on pollen deposition in strawberry showed that 4 bee visits provides full pollen deposition for a strawberry flower (Chagnon et al. 1989, MacInnis & Forrest 2019). So, to ensure adequate pollen quantity, the HB treatment consisted of 5 consecutive visits by honey bees to a flower, and the WB treatment consisted of 5 consecutive visits by wild bees. The two bee treatments, (HB and WB), were replicated as many times as possible in each plot (SS, ALB, MULT) each day.

To obtain the bee treatments, one secondary unopened flower on a single plant in each plot was haphazardly chosen, covered with ‘no-see-um’ nylon mesh (BioQuip, CA, USA) to exclude pollinators, and given a blank label. Secondary flowers were used because they are more abundant than primary flowers and more likely to set fruit than those flowering later in the inflorescence hierarchy. When ready, an observer would unbag a labelled flower and watch the flower until a HB or WB visited. The flower was then assigned a treatment based on the identity of the first visitor. For example, if a WB was the first to enter one of the sampled flowers, all HBs that subsequently approached the flower would be waved away. It was also noted whether each bee approached the focal flower from within the same row (within-row) or not. We could only reliably categorize bees that originated within the same row, as most (especially small) WB visitors coming from elsewhere appeared in the flowers too quickly to determine their exact origin. Consequently, all bees that did not appear to originate within the same row were classified as “unknown” origin. When a WB visited a sample flower, a large (46 cm diameter) net was carefully placed over the whole plant with the top of the net held well above the plant so as not to disturb the visitor. Once the bee finished foraging she would typically fly upward into the top of the net where she was caught and later identified to species. Bees were identified to species using the keys developed by Ascher and Pickering (2017); bees in the genus *Lasioglossum* were identified to species using Gibbs (2011); Gibbs et al. (2013). Once a treatment was completed (5 visits of one bee type per flower), the sampled flower was covered with a mesh bag for two days or until stigmas browned. We attempted to perform an equal number of bee treatments in each plot (ALB, SS, MULT) each day, but this was not possible due to variability in flower visit frequencies, resulting in unequal sample sizes between plots and bee types. Beginning two weeks after each pollination treatment, strawberry development of the treated flowers was monitored each day. Individual strawberries were harvested when they ripened (i.e., when they were bright red and easily detachable from the pedicel). All strawberries were harvested in the morning, 17–24 days post-pollination, and were weighed on an analytical balance within two hours of harvesting to minimize water loss.

We also implemented positive and negative control treatments (i.e., hand-cross-pollinated and autonomously self-pollinated flowers) within each plot. For the cross-pollinated treatments, in each of the single-variety and multiple-variety plots (two plots per block), five ALB flower buds on five different plants were bagged with pollinator-exclusion mesh until open and subsequently pollinated with SS pollen. The pollen was collected from a combination of 10 SS flowers from 10 different plants by shaking the flower and pollen into a small petri dish. The pollen mixture was then immediately brushed onto the flower stigmas with a small paintbrush. In the same manner, five SS flowers in the single- and multiple-variety plots were cross-pollinated by hand with ALB pollen. All control flowers were re-bagged with mesh until the end of receptivity (petals abscised and stigmas browned) to exclude pollinator visits. This resulted in 20 cross-pollinated flowers per variety and row configuration. These hand-cross-pollinated flowers were used as a baseline against which to compare the cross-pollination effectiveness of each bee type (HB and WB).

For the self-pollination treatment, 25 flowers each of SS, ALB, and MULT were bagged with mesh from the bud stage to petal abscission to exclude pollinators. In the bud stage, five flowers from five plants of each variety (SS, ALB) and field design (single- and multiple-variety) were bagged with pollinator-exclusion mesh in each plot. The bag was removed once stigmas were no longer receptive (petal abscission, stigmas browned) and flowers were monitored until fruit development. This treatment was used to determine the fruit mass for each variety in the absence of pollinators (autonomous self-pollination).

4.3.2 Statistical analyses

All statistical analyses were conducted with R v.3.3.3 (R Core Team, 2017). To investigate the effects of pollination treatment (WB, HB, cross-pollinated, self-pollinated), field design (single-variety, multiple variety) and variety (SS, ALB) on berry mass we used a linear mixed-effects model (LMM; lme4 package, Bates et al. 2015) fitted with restricted maximum likelihood (REML) due to the unbalanced design (Bolker et al. 2008). Model assumptions (homogeneity of variance, linearity, and normality of residuals) were tested using Levene’s test for homogeneity of variance (package: car; Fox & Weisberg 2011) and visual inspection of residual plots. Berry mass was the response variable, and field design, variety, pollination treatment, and all interactions among these variables were fixed effects. Plant ID nested within plot and block was included as a random factor in the model, as some plants had two treated flowers over the sampling period. Sampling date was also included as a fixed continuous variable to account for the influence of plant age, and other environmental variables that may have changed directionally through the season, on strawberry plant growth and fruit development. The effects of pollination treatment and field design on strawberry mass were analysed separately for each variety with the 'phia' package (De Rosario-Martinez 2015), designed to analyse interaction contrasts for mixed-effects models. Specifically, we tested the effects of bee pollination treatments (HB, WB) in both field designs (single-variety, multiple-variety) on strawberry mass, then we compared the bee pollination treatments to the cross-pollinated treatments in each field design.

4.4 Results

4.4.1 Bee community

Weather conditions permitted 42 days (336 h) of sampling over the 66 day blooming period. The number of flowers that received the 5 bee visits required varied each day (6.6 ± 3.9 flowers/day), potentially due to the attractiveness of other crops flowering nearby. Herbivory damage further reduced the sample size to a total of 156 flowers (780 bee visits) suitable for subsequent yield analyses. Honey bees (*Apis mellifera* L.) comprised 380 visits and wild bees from 8 genera and 22 species comprised 400 visits (see Table 4.1 for full species list). The wild bee community was dominated by bees in the genera *Lasioglossum* (n = 363 visits), *Augochlora* (n = 27), and *Halictus* (n = 6). Only one male bee was caught over the study period, so we considered only females in our analysis.

4.4.2 Fruit mass, field design, and foraging behaviour

The effect of field design (single or multiple variety) on strawberry mass depended on pollination treatment and strawberry variety (significant pollination treatment × field design × variety interaction; $\chi^2_3 = 10.40$, $p = 0.015$). In the multiple-variety fields, WB-visited ALB flowers produced strawberries that were significantly larger (mean ± SD, 24.05 ± 3.61 g) than HB-visited flowers (16.38 ± 7.45 g, $\chi^2_1 = 14.52$, $p < 0.001$; Fig. 4.2; Table 4.2). WB-visited ALB flowers in multiple-variety fields also yielded significantly larger fruit than WB-visited ALB flowers in single-variety fields (19.47 ± 4.06 g, $\chi^2_1 = 9.35$, $p = 0.009$; Table 4.2). HB-visited ALB flowers in multiple-variety fields (16.38 ± 7.45 g) did not differ in mass from HB-visited ALB strawberries in single-variety fields (15.41 ± 5.80 g, $\chi^2_1 = 1.06$, $p = 0.31$; Table 4.2). There were no significant differences in strawberry mass between HB-pollinated (15.41 ± 5.80 g) and WB-pollinated ALB flowers (19.47 ± 4.06 g) in the single-variety plots ($\chi^2_1 = 2.45$, $p = 0.12$). SS flowers produced strawberries of the same mass regardless of bee identity in the multiple-variety plots ($\chi^2_1 = 2.32$, $p = 0.13$; Fig. 4.2; Table 4.2) and in the single variety plots ($\chi^2_1 = 3.74$, $p = 0.11$).

There were significant differences in the pre-visit origin (within-row vs. unknown) between bee types ($\chi^2_1 = 258.89$, $p < 0.001$). Out of 380 HB visits, 318 originated within the same row (84%), whereas only 108 out of 400 of wild bee visits visibly originated within the same row (27%). However, as it was difficult to track the location of the wild bees before they landed in the sample flower, so this may be a conservative estimate of the fraction of wild bees that came from the same row.

4.4.3 Cross-pollinated and autonomously self-pollinated controls

Contrasting the cross-pollinated control flowers with the bee-pollinated flowers revealed that honey bee-pollinated ALB strawberries weighed less (15.41 ± 5.79 g) than cross-pollinated strawberries in single-variety plots (23.40 ± 3.86 g, $\chi^2_1 = 15.84$, $p < 0.001$), as did wild bee-pollinated strawberries ($\chi^2_1 = 5.63$, $p = 0.035$; Fig. 4.3a; Table 4.3). However, only the honey bee-pollinated strawberries weighed less than the cross-pollinated strawberries in multiple-variety ALB plots ($\chi^2_1 = 4.45$, $p = 0.034$; Fig. 4.3b; Table 4.3); wild bee-pollinated strawberries in these plots actually weighed marginally more than hand-crossed strawberries ($\chi^2_1 = 2.74$, $p = 0.098$; Table 4.3). Autonomously self-pollinated flowers produced ALB strawberries that were smaller than both HB- and WB-pollinated strawberries in the single-variety (Fig. 4.3a; Table 4.4) and multiple-variety plots (Fig. 4.3b; Table 4.4). There was no significant difference in SS strawberry mass among pollination types (WB, HB, crossed, selfed) in the single-variety plots (Fig. 4.3c; Table 4.4) or in the multiple-variety plots (Fig. 4.3d; Table 4.4).

4.5 Discussion

The configuration of plants and varieties within the field has the potential to influence the type of pollen (self vs. outcrossed) transferred between crop plants (Levin 1979; Kumar et al. 2013). Our results show that bee identity significantly influenced strawberry mass in experimental fields inter-planted with two strawberry varieties. Hand-cross-pollinated flowers and wild bee-pollinated flowers were of similar mass in multiple-variety plots, suggesting that wild bees transferred more outcrossed pollen to *Albion* plants in the multiple-variety plots than did honey bees. However, the greater strawberry mass of wild bee-visited flowers was specific to the *Albion* variety: strawberries of the more self-compatible *Seascape* variety (Bringhurst & Voth 1991) attained a similar mass, regardless of pollinator identity (Fig. 4.2). This highlights the effect of variety on fruit characteristics, irrespective of the influence of field design and pollinator foraging behaviour.

The positive influence of wild bees on strawberry mass seen in the *Albion* variety was largely driven by bees in the genus *Lasioglossum*, the dominant wild pollinators in this community. Our results suggest that, unlike honey bees, *Lasioglossum* bees tend to move between rows rather than within rows, as 73% did not visibly originate within the row, whereas 85% of honey bees did. Therefore, the wild bees in this community are more likely than managed honey bees to achieve cross-pollination, provided suitable outcross pollen is available in adjacent rows. It is unlikely that differences between bee types in the quantity of pollen deposited were responsible for differences in yield, as previous work showed no differences in the quantity of pollen deposited among bee species (MacInnis & Forrest 2019), and five bee visits should have been more than sufficient to saturate stigmas.

Honey bees characteristically forage in a straight line to the nearest-neighbouring plant or flower (Waddington 1980; Ginsberg 1986; Morris 1993; Walters & Schultheis 2009). In an agricultural environment, this strategy means that honey bees typically move within, rather than between crop rows, unless forced to deviate from this pattern by interactions with other insects (DeGrandi-Hoffman & Watkins 2000; Greenleaf & Kremen 2006; Brittain et al. 2013). As honey bees were abundant on site, competition with honey bees may have influenced wild bee foraging behaviour in this study. We observed that smaller bees were more prone to adjust foraging behaviour (i.e., leave a flower) when encountering honey bees, which may have resulted in an increase in the distance between plants visited by small wild bees. Furthermore, a forager that increases the distance travelled between flower visits lowers the probability of visiting a previously-visited flower (Zimmerman 1979). Because managed pollinators were abundant within the relatively small experimental field site (~ 4000 m^2), the probability of visiting a previously-visited strawberry flower may have been high. In this context, the abundant honey bee population may have forced wild bees to travel long distances between visited plants. Further research is needed to gain more insight on the influence of interspecies interactions on pollinator movement in strawberry.

The relationship between pollinator movement and pollen transport is complex, and the connection between the two can be difficult to establish. Pollen transport is affected not only by pollinator movement patterns, but also by inter- and intraspecific differences in pollination efficiency and pollen carryover. Bees can vary in pollination efficiency for a variety of reasons, including their inherent pollen-carrying capacity, the degree to which they groom pollen from their bodies, and their within-flower foraging behaviours. Pollen carryover occurs when flowers receive pollen from several flowers, not just the last flower visited (Price & Waser 1979; Thomson & Plowright 1980); this can cause gene flow to exceed the average distance travelled by foragers (Schaal 1980; Levin 1981; Karron et al. 1995). Pollen carryover can also be affected by the degree of grooming between and during flower visits (Rademaker et al. 1997; Holmquist et al. 2012). The positive effect of *Lasioglossum* pollination on strawberry yield could have resulted from a combination of high pollen carryover and a low degree of directionality in our experimental fields. Experiments using dyes (e.g. Thomson et al. 1986; Adler & Irwin 2006) or genes (e.g. Ellstrand et al. 1989, Kohn & Casper 1992) to track pollen movement would be needed to investigate this possibility further.

Emerging studies are finding wild bees to be more effective pollinators than the European honey bee for many crops, including strawberry (Hoehn et al. 2008; Holzschuh et al. 2012;

Garibaldi et al. 2013; Rogers et al. 2014; Mallinger & Gratton 2014; Horth & Campbell 2018; MacInnis & Forrest 2019; Castle et al. 2019), and interspecific differences in foraging behaviour are an important factor driving this effect (DeGrandi-Hoffman & Watkins 2000; Greenleaf & Kremen 2006; Brittain et al. 2013). If the low degree of directionality observed in *Lasioglossum* bees here is consistent at the farm scale, this behaviour could be utilized to increase strawberry yield (particularly for varieties with limited self-compatibility) when coupled with an increase in varietal diversity on the farm.

4.5.1 Conclusions

Research on the influence of foraging behaviour on cross-pollination in crops is limited. Further studies are needed to determine whether the foraging behaviour of the bees observed here is consistent in other contexts, on larger scales and for other strawberry varieties. If particular species of wild bees are found to consistently travel between rather than within rows, designing the farmed landscape with pollinator foraging patterns in mind may benefit crop yields. Incorporating multiple varieties in close proximity may increase cross-pollination by wild bee species, and potentially by managed honey bees if multiple varieties are planted within-row—a possibility that was not explored here. Currently, many strawberry farmers in our area plant multiple varieties per field based on proven performance and consumer preferences (G. MacInnis, pers. communication with growers). However, each variety is typically planted in ten or more adjacent rows. Strawberries are either planted by hand directly into the ground as a mechanical transplanter moves along the rows, or they are fed manually into transplant slots. Therefore, alternating varieties by row would simply be a matter of positioning the seedlings with the plant handlers accordingly, and could likely be implemented without additional costs or labour.

Table 4.1: Bee species observed in the experimental strawberry plots in Southern Quebec in 2017.

Family	Genus	Species	No. individuals
Apidae	*Apis*	*mellifera* Linnaeus	380
Halictidae	*Lasioglossum*	*ellisiae* (Sandhouse)	197
Halictidae	*Lasioglossum*	*tegulare* (Robertson)	113
Halictidae	*Augochlora*	*pura* (Say)	27
Halictidae	*Lasioglossum*	*pilosum* (Smith)	20
Halictidae	*Lasioglossum*	*lineatulum* (Crawford)	10
Halictidae	*Lasioglossum*	*versans* (Lovell)	9
Halictidae	*Lasioglossum*	*leucocomum* (Lovell)	5
Halictidae	*Halictus*	*confusus* Smith	2
Halictidae	*Halictus*	*ligatus* (Say)	2
Halictidae	*Halictus*	*rubicundus* Christ	2
Halictidae	*Lasioglossum*	*viridatum* (Robertson)	2
Halictidae	*Agapostemon*	*texanus* Cresson	1
Apidae	*Bombus*	*impatiens* Cresson	1
Apidae	*Ceratina*	*dupla* (Say)	1
Megachilidae	*Hoplitis*	*producta* (Cresson)	1
Halictidae	*Lasioglossum*	*foveolatum* (Robertson)	1
Halictidae	*Lasioglossum*	*imitatum* (Smith)	1
Halictidae	*Lasioglossum*	*mitchelli* Gibbs	1
Halictidae	*Lasioglossum*	*novascotiae* (Mitchell)	1
Halictidae	*Lasioglossum*	*subversans* (Mitchell)	1
Halictidae	*Lasioglossum*	*subviridatum* (Cockerell)	1
Halictidae	*Lasioglossum*	*succinipenne* (Ellis)	1

Table 4.2: Results of LMMs of strawberry mass (mass per strawberry) by field design and bee type. The interaction between bee identity and field design is subdivided into two orthogonal contrasts for each strawberry variety. Results of the comparisons of bee type in each field design for each variety are also listed. n is the number of strawberries for each group. Significant differences are indicated in bold.

Variety	Bee/field type	Contrast	Mean ± SD (g)	n	χ^2	p
Albion	Honey bee	Single-variety vs.	15.41 ± 5.80	11		
		Multiple-variety	16.38 ± 7.45	14	1.06	0.31
	Wild bee	Single-variety vs.	18.80 ± 4.25	15		
		Multiple -variety	24.05 ± 3.61	12	**9.35**	**0.009**
	Single-variety	Honey bee vs.	15.41 ± 5.80	11		
		Wild bee	19.47 ± 4.06	15	2.45	0.12
	Multiple-variety	Honey bee vs.	16.38 ± 7.45	14		
		Wild bee	24.05 ± 3.61	12	**14.52**	**<0.001**
Seascape	Honey bee	Single-variety vs.	17.30 ± 5.29	30		
		Multiple-variety	16.27 ± 6.22	21	2.74	0.30
	Wild bee	Single-variety vs.	18.62 ± 4.05	18		
		Multiple-variety	18.14 ± 4.70	35	3.14	0.24
	Single-variety	Honey bee vs.	17.30 ± 5.29	30		
		Wild bee	18.62 ± 4.05	18	3.74	0.11
	Multiple-variety	Honey bee vs.	16.27 ± 6.22	21		
		Wild bee	18.14 ± 4.70	35	2.32	0.13

Table 4.3: Results of the comparisons of the mass of strawberries (Strawberry mass) pollinated by each bee type (Honey bee, wild bee) to the hand cross-pollinated controls (Cross-pollinated). Single-variety Albion in plots that contained only strawberry plants of the Albion variety. Multiple-variety Albion plots had both Albion and Seascape plants in alternating rows. Single-variety Seascape are plots that contained only strawberry plants of the Seascape variety. Multiple-variety Seascape plots had both Seascape and Albion plants in alternating rows. n is the number of strawberries for each group, χ^2 is the test statistic, and p is the significance. Significant differences are indicated in bold.

	Strawberry mass Mean ± SD (g)	n	χ^2	p
Single-variety Albion				
Cross-pollinated	23.40 ± 3.86	14		
Honey bee	15.41 ± 5.79	11	15.84	**<0.001**
Wild bee	18.80 ± 4.25	15	5.63	**0.035**
Multiple-variety Albion				
Cross-pollinated	21.48 ± 4.63	15		
Honey bee	16.38 ± 7.44	14	4.45	**0.034**
Wild bee	24.05 ± 3.60	12	2.74	0.098
Single-variety Seascape				
Cross-pollinated	15.57 ± 2.82	13		
Honey be	17.30 ± 5.29	30	1.79	0.36
Wild bee	18.61 ± 4.05	18	3.74	0.11
Multiple-variety Seascape				
Cross-pollinated	17.24 ± 3.48	16		
Honey bee	16.27 ± 6.22	21	0.11	0.73
Wild bee	18.15 ± 4.70	35	2.32	0.13

Table 4.4: Results of the comparisons of the mass of strawberries (Strawberry mass) pollinated by each bee type (Honey bee, Wild bee) to the autonomously self-pollinated controls (Selfed). Single-variety Albion are plots that contained only strawberry plants of the Albion variety. Multiple-variety Albion plots had both Albion and Seascape plants in alternating rows. Single-variety Seascape in plots that contained only strawberry plants of the Seascape variety. Multiple-variety Seascape plots had both Seascape and Albion plants in alternating rows. n is the number of strawberries for each group, χ^2 is the test statistic, and p is the significance. Significant differences are indicated in bold.

Single-variety Albion	**Strawberry mass Mean ± SD (g)**	**n**	**χ^2**	**p**
Selfed	11.19 ± 4.79	22		
Honey bee	15.41 ± 5.79	11	6.41	**0.031**
Wild bee	19.47 ± 4.06	15	23.36	**<0.001**
Multiple-variety Albion				
Selfed	12.45 ± 4.28	23		
Honey bee	16.38 ± 7.44	14	7.33	**0.013**
Wild bee	24.05 ± 3.60	12	45.75	**<0.001**
Single-variety Seascape				
Selfed	16.82 ± 4.53	19		
Honey bee	17.30 ± 5.29	30	0.36	0.92
Wild bee	18.61 ± 4.05	18	3.34	0.14
Multiple-variety Seascape				
Selfed	17.51 ± 4.55	21		
Honey bee	16.27 ± 6.22	21	0.46	0.94
Wild bee	18.15 ± 4.70	35	1.01	0.31

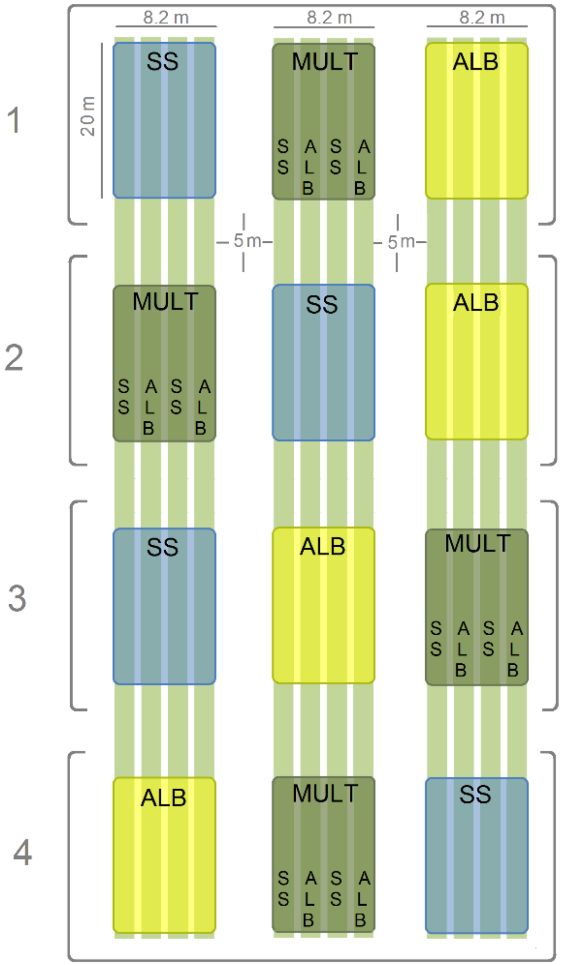

Figure 4.1. Experimental design. Field plots were arranged in a randomized complete block design with each plot containing four rows of strawberry plants 20 m long and 8.2 m wide. Numbers at left refer to the four experimental blocks. 'SS' indicates plots containing only strawberry plants of the Seascape variety in each of the 4 rows. 'ALB' indicates plots containing only strawberry plants of the Albion variety. 'MULT' plots contained two rows of Albion and two rows of Seascape interplanted within the plot

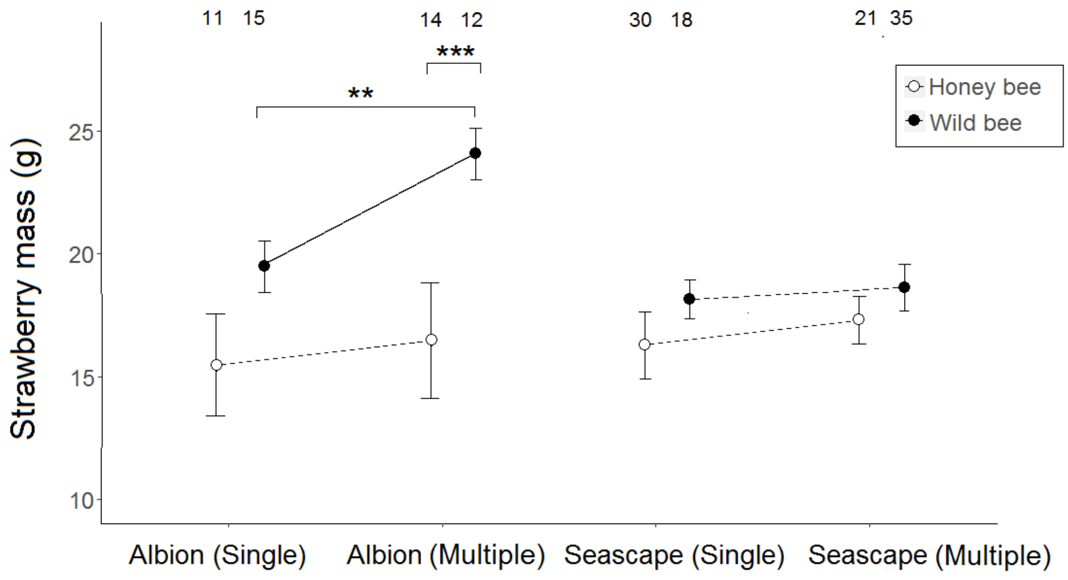

Figure 4.2. Interactive effects of bee type and field design on the mass of two strawberry varieties. 'Albion (Single)' were strawberries in plots that contained only plants of the Albion variety. 'Albion (Multiple)' indicates Albion strawberries in plots that had Albion and Seascape plants in alternating rows. 'Seascape (Single)' were strawberries in plots that contained only strawberry plants of the Seascape variety. 'Seascape (Multiple)' were plots that contained Albion and Seascape plants in alternating rows. Open circles indicate strawberries that developed from honey bee-pollinated flowers and closed circles indicate strawberries that developed from wild bee-pollinated flowers. Error bars represent the standard error. Sample sizes are listed above. Solid line connecting symbols indicates $p < 0.05$; dashed lines indicate no significant difference between field designs. Asterisks indicate significant differences within field designs and bee types; ** = $p < 0.01$, *** = $p < 0.001$.

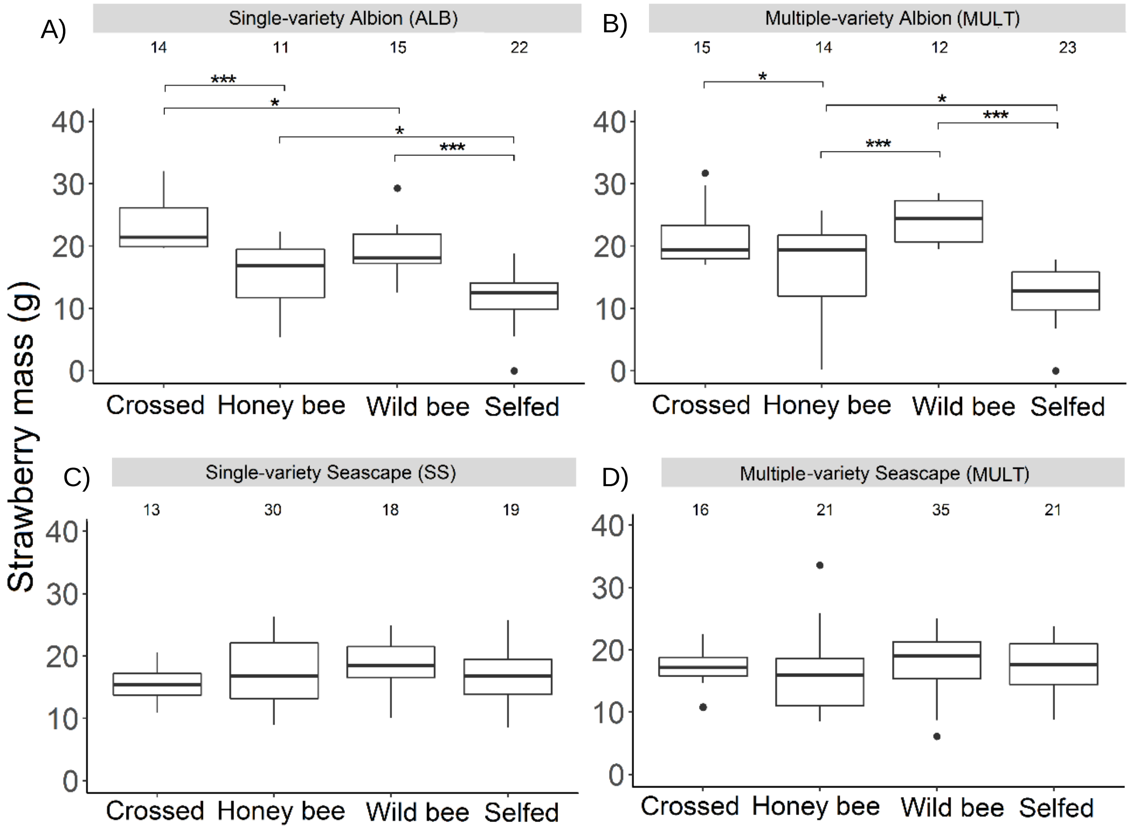

Figure 4.3. Mean mass of strawberries produced by honey bee- and wild bee-pollinated flowers in each field type compared with the hand-cross-pollinated ("Crossed") and bagged ("Selfed") controls. 'Single-variety Albion (ALB)' were strawberries of the Albion variety in plots that contained only Albion plants. 'Multiple-variety Albion (MULT)' indicates Albion strawberries in plots that had both Albion and Seascape plants in alternating rows. 'Single-variety Seascape (SS)' were strawberries in plots that contained only strawberry plants of the Seascape variety. 'Multiple-variety Seascape (MULT)' were Seascape strawberries in plots that contained both Albion and Seascape plants in alternating rows. Sample sizes are listed above. Asterisks indicate significant differences, * = $p < 0.05$, *** = $p < 0.001$.

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4.7 References

Adler, L.S., Irwin, R.E., 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Ann. Bot. 97, 141–150. https://doi.org/10.1093/aob/mcj012

Ascher, J. S., and Pickering, J. 2017. Discover Life Bee Species Guide and World Checklist (Hymenoptera: Apoidea: Anthophila). Retrieved from http://www.discoverlife.org/mp/20q?guide=Apoidea_species

Barrett, S.C.H., 2003. Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 358, 991–1004. https://doi.org/10.1098/rstb.2003.1301

Bates, D., Maechler, M., Bolker, B., Steve Walker. 2015. Fitting linear mixed-effects models using lme4. J. Statistical Softw. 67(1), 1-48. doi:10.18637/jss.v067.i01.

Blitzer, E.J., Gibbs, J., Park, M.G., Danforth, B.N., 2016. Pollination services for apple are dependent on diverse wild bee communities. Agric. Ecosyst. Environ. 221, 1–7. https://doi.org/10.1016/j.agee.2016.01.004

Bolker, B. M., 2008. *Ecological Models and Data in R*. Princeton University Press.

Bommarco, R., Marini, L., Vaissière, B. E. 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. Oecologia, 169(4), 1025-1032.

Bringhurst, R. S., Voth, V. United States Patent No. US07479499. Retrieved from https://patentimages.storage.googleapis.com/a3/80/bf/8121297cc7a88d/USPP7614.pdf

Brittain, C., Williams, N., Kremen, C., Klein, A., 2013. Synergistic effects of non-apis bees and honey bees for pollination services. Proc. Biol. Sci. 280, 2012.2767. https://doi.org/10.1098/rspb.2012.2767

Cane, J.H., Schiffhauer, D., Kervin, L.J., 1996. Pollination, foraging, and nesting ecology of the leaf-cutting bee Megachile (Delomegachile) addenda (Hymenoptera: Megachilidae) on Cranberry Beds, Ann. Entomol. Soc. Am. 89, 61-7.

Castle, D., Grass, I., Westphal, C. 2019. Fruit quantity and quality of strawberries benefit from enhanced pollinator abundance at hedgerows in agricultural landscapes. 2019. Agric. Ecosyst. Environ. 275(1). 14-22. https://doi.org/10.1016/j.agee.2019.01.003

Chagnon, M., Gingras, J., De Oliveira, D., 1989. Effect of honey bee (Hymenoptera: Apidae) visits on the pollination rate of strawberries. J. Econ. Entomol. 82, 1350–1353. https://doi.org/10.1093/jee/82.5.1350

Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression and its evolutionary consequences, Ann. Rev. Ecol. Syst. 18. 237-268.

Colbert, S., and de Oliveira, D., 1992. Cross-pollination and production of 4 cultivars of strawberry, *Fragaria x ananassa*. Can. J. Plant Sci. 72, 857-861.

Cranmer, L., McCollin, D., Ollerton, J., 2012. Landscape structure influences pollinator movements and directly affects plant reproductive success. Oikos 121, 562–568. https://doi.org/10.1111/j.1600-0706.2011.19704.x

Cresswell, J.E., Osborne, J.L., Bell, S.A., 2002. A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. Oikos 98, 375–384. https://doi.org/10.1034/j.1600-0706.2002.980302.x

Darwin, C. R. 1876. The Effects Of Cross And Self Fertilisation In The Vegetable Kingdom. London: John Murray.

DeGrandi-Hoffman, G., Watkins, J.C., 2000. The foraging activity of honey bees *Apis mellifera* and non—Apis bees on hybrid sunflowers (*Helianthus annuus*) and its influence on cross—pollination and seed set. J. Apic. Res. 39. 37–45. https://doi.org/10.1080/00218839.2000.11101019

de Melo e Silva Neto, C., Lima, F. G., Gonçalves, B. B., Bergamini, L. L., Bergamini, B. A. R., Elias, M. D. S., and Franceschinelli, E. V. 2013. Native bees pollinate tomato flowers and increase fruit production. J. Pollin. Ecol. 11(6), 41-45.

Denney, J. O. 1992. Xenia includes metaxenia. HortScience. 27(7), 722-728.

de Oliveira D., Pion S., Paradis R.O. 1983. Entomogamie et production du fraisier "Redcoat," *Fragaria* x *ananassa* Duch., au Quebec. Bui Soc Entomol Fr 88(2):356-359.

De Rosario-Martinez, H. 2015. Phia: Post-Hoc Interaction Analysis. R Package Version 0.2–1.

Ellstrand, N.C., Devlin, B., Marshall, D.L., 1989. Gene flow by pollen into small populations: data from experimental and natural stands of wild radish. Proc. Natl. Acad. Sci. U. S. A. 86, 9044–7. https://doi.org/10.1073/PNAS.86.22.9044

Forrest, J.R.K., Thorp, R.W., Kremen, C., Williams, N.M., 2015. Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape. J. Appl. Ecol. 52(3). 706–715. https://doi.org/10.1111/1365-2664.12433

Fox, J., Weisberg, S. 2011. An {R} Companion to Applied Regression, second edition. Sage; Thousand Oaks CA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Free, J.B., 1962. The effect of distance from pollinizer varieties on the fruit set on trees in plum and apple orchards. J. Hortic. Sci. 37, 262–271. https://doi.org/10.1080/00221589.1962.11514045

Free, J.B., 1968. The pollination of strawberries by honey-bees. J. Hortic. Sci. 43, 107–111.

https://doi.org/10.1080/00221589.1968.11514237

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., …, Klein, A.M. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science. 339, 1608–11. https://doi.org/10.1126/science.1230200

Gibbs, J., 2011. Revision of the metallic *Lasioglossum* (*Dialictus*) of eastern North America (Hymenoptera: Halictidae: Halictini), Zootaxa. 3073, 1-216.

Gibbs, J., Packer, L., Dumesh, S., Danforth, B.N., 2013. Revision and reclassification of *Lasioglossum* (*Evylaeus*), *L.* (*Hemihalictus*) and *L.* (*Sphecodogastra*) in eastern North America (Hymenoptera: Apoidea: Halictidae), Zootaxa. https://doi.org/10.11646/zootaxa.3672.1.1

Ginsberg, H., 1986. Honey bee orientation behaviour and the influence of flower distribution on foraging movements. J. Econ. Entomol. 11, 173–179. https://doi.org/10.1111/j.1365-2311.1986.tb00292.x

Goulson, D., 2000. Why do pollinators visit proportionally fewer flowers in large patches? Oikos 91, 485–492. https://doi.org/10.1034/j.1600-0706.2000.910309.x

Greenleaf, S.S., Kremen, C., 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. PNAS U. S. A. 103, 13890–5. https://doi.org/10.1073/pnas.0600929103

Handel, S. N. (1983). Pollination ecology, plant population structure, and gene flow. Pollinat. Biology. 163, 211.

Harder, L.D., Barrett, S.C.H., 1996. Pollen dispersal and mating patterns in animal-pollinated plants, in: Floral Biology. Springer US, Boston, MA, 140–190. https://doi.org/10.1007/978-1-4613-1165-2_6

Heinrich, B., 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. Oecologia 40, 235–245. https://doi.org/10.1007/BF00345321

Herrera, C.M., 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos 50, 79–90. https://doi.org/10.2307/3565403

Hoehn, P., Tscharntke, T., Tylianakis, J.M., Steffan-Dewenter, I., 2008. Functional group diversity of bee pollinators increases crop yield. Proc. Biol. Sci. 275, 2283–91. https://doi.org/10.1098/rspb.2008.0405

Holmquist, K.G., Mitchell, R.J., Karron, J.D., 2012. Influence of pollinator grooming on pollen-mediated gene dispersal in *Mimulus ringens* (Phrymaceae). Plant Species Biol. 27, 77–85. https://doi.org/10.1111/j.1442-1984.2011.00329.x

Holmquist, K. G., Mitchell, R. J., and Karron, J. D. 2012. Influence of pollinator grooming on pollen-mediated gene dispersal in *Mimulus ringens* (Phrymaceae). Plant Spec. Biol. 27(1), 77-85.

Holzschuh, A., Dudenhöffer, J. H., and Tscharntke, T. 2012. Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biol. Conserv. 153, 101-107.

Horth, L., Campbell, L.A., 2017. Supplementing small farms with native mason bees increases strawberry size and growth rate. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.12988

Ivey, C.T., Martinez, P., Wyatt, R., 2003. Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae). Am. J. Bot. 90, 214–225. https://doi.org/10.3732/ajb.90.2.214

Institut de la statistique du Québec (ISQ) - La Financière agricole du Québec, 2017. Strawberry Crop Conditions. Accessed at http://www.stat.gouv.qc.ca/statistiques/agriculture/etat-cultures/septembre2017/fraises.htm

Javorek, S.K., Mackenzie, K.E., Kloet, S.P. Vander, 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Ann. Entomol. Soc. Am. 95, 345–351. https://doi.org/10.1603/0013-8746(2002)095[0345:cpeabh]2.0.co;2

Karron, J.D., Thumser, N.N., Tucker, R., Hessenauer, A.J., 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity. 75, 175–180. https://doi.org/10.1038/hdy.1995.121

Karron, J.D., Holmquist, K.G., Flanagan, R.J., Mitchell, R.J., 2009. Pollinator visitation patterns strongly influence among-flower variation in selfing rate. Ann. Bot. 103, 1379–1383. https://doi.org/10.1093/aob/mcp030

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., Tscharntke, T. 2014. Bee pollination improves crop quality, shelf life and commercial value. Proc. Biol. Sci., 281(1775), 20132440.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. Biol. Sci. 274, 303–13. https://doi.org/10.1098/rspb.2006.3721

Kohn, J. R., and Casper, B. B. 1992. Pollen-mediated gene flow in *Cucurbita foetidissima* (Cucurbitaceae). Am. J. Bot. 79(1), 57-62. https://doi.org/10.1002/j.1537-2197.1992.tb12623.x

Kumar, R., Dia, M., and Wehner, T. C. 2013. Implications of mating behavior in watermelon breeding. HortScience, 48(8), 960-964.

Levin, D.A., Kerster, H.W., 1969. The dependence of bee mediated pollen and gene dispersal on plant density. Evolution. 23, 560-572. https://doi.org/10.1111/j.1558-5646.1969.tb03541.x

Levin D.A. 1979. Pollinator foraging behavior: genetic implications for plants. In: Solbrig O.T., Jain S., Johnson G.B., Raven P.H. (eds) *Topics in Plant Population Biology*. Palgrave, London

Levin, D.A., 1981. Dispersal versus gene flow in plants. Ann. Missouri Bot. Gard. 68. 233-253. https://doi:10.2307/2398797.

Lloyd, D.G., Schoen, D.J., 1992. Self- and cross-fertilization in plants. I. Functional dimensions. Int. J. Plant Sci. 153, 358–369. https://doi.org/10.1086/297040

MacInnis, G., and Forrest, J. R. K. 2019. Pollination by wild bees yields larger strawberries than pollination by honey bees. J. Appl. Ecol. 56(4), 824-832. https://doi.org/10.1111/1365-2664.13344

Mallinger, R.E. and Gratton, C. 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. J. Appl. Ecol. 52, 323–330. https://doi.org/10.1111/1365-2664.12377

Mandelik, Y., Winfree, R., Neeson, T., Kremen, C., 2012. Complementary habitat use by wild bees in agro-natural landscapes. Ecol. Appl. 22, 1535–1546. https://doi.org/10.1890/11-1299.1

Morandin, L. A., Laverty, T. M., Kevan, P. G. 2001. Effect of bumble bee (Hymenoptera: Apidae) pollination intensity on the quality of greenhouse tomatoes. J. Econ. Entomol. 94(1), 172-179.

Morris, W.F., 1993. Predicting the consequence of plant spacing and biased movement for pollen dispersal by honey bees. Ecology. 74, 493–500. https://doi.org/10.2307/1939310

Ontario ministry of Agriculture, Food and Rural Affaris (OMAFRA). 2016. Dayneutral Strawberries. Production Factsheet. Accessed April 2017: http://www.omafra.gov.on.ca/english/crops/facts/89-099.htm

Price, M. V., and Waser, N. M. (1979). Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature. 277(5694), 294-297. https://doi.org/10.1038/277294a0

Pyke, G.H., 1978. Optimal foraging: Movement patterns of bumblebees between inflorescences. Theor. Popul. Biol. 13, 72–98. https://doi.org/10.1016/0040-5809(78)90036-9

Pyke, G.H., Cartar, R. V., 1992. The Flight Directionality of Bumblebees: Do They Remember Where They Came from?, Oikos 65, 321. https://doi.org/10.2307/3545025

R Core Team, 2017. R: A Language And Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL:http://www.Rproject.org/.

Rademaker, M.C.J., Jong, T.J. De, Klinkhamer, P.G.L., n.d. Pollen Dynamics of Bumble-Bee Visitation on *Echium vulgare*. Funct. Ecol. https://doi.org/10.2307/2390395

Raw, A., 2000. Foraging behaviour of wild bees at hot pepper flowers (*Capsicum annuum*) and its possible influence on cross pollination. Ann. Bot. 85, 487–492. https://doi.org/10.1006/anbo.1999.1090

Rogers, S. R., Tarpy, D. R., and Burrack, H. J. 2014. Bee species diversity enhances productivity and stability in a perennial crop. PLoS One, 9, e97307. https://doi.org/10.1371/journal.pone.0097307

Routley, M.B., Mavraganis, K., Eckert, C.G., 1999. Effect of population size on the mating system in a self-compatible, autogamous plant, *Aquilegia canadensis* (Ranunculaceae). Heredity. 82, 518–528. https://doi.org/10.1038/sj.hdy.6885220

Sarracino, J.M., Vorsa, N., 1991. Self and cross fertility in cranberry. Euphytica. 58, 129–136. https://doi.org/10.1007/BF00022813

Schaal, B. A. 1980. Measurement of gene flow in *Lupinus texensis*. Nature. 284(5755). 450-451. https://doi.org/10.1038/284450a0

Schmitt, J. 1983. Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in *Linanthus bicolor*. Evolution. 37. 1247-1257.

Serrano, A. R., Guerra-Sanz, J. M., 2006. Quality fruit improvement in sweet pepper culture by bumblebee pollination. Sci. Hortic., 110(2), 160-166. https://doi.org/10.1016/J.SCIENTA.2006.06.024

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. 12(1), 253-279.

Shaw, D.V., Larson, K.D., 2006. United States Patent No. USPP16228P3. Retrieved from https:// https://patentimages.storage.googleapis.com/61/ce/e7/aca66ac422372b/USPP16228.pdf

Swingle W.T., 1928. Metaxenia in the date palm. J. Hered. 19, 257-268.

Thomson, J. D., and Plowright, R. C. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. Oecologia, 46(1), 68-74. https://doi.org/10.1007/BF00346968

Thomson, J. D., Price, M. V., Waser, N. M., and Stratton, D. A. 1986. Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*. Oecologia. 69(4), 561-566. https://doi.org/10.1007/BF00410363

Thomson, J. D., and Thomson, B. A. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. Evolution, 43(3), 657-661. https://doi.org/10.1111/j.1558-5646.1989.tb04261.x

Tuohimetsä, S., Hietaranta, T., Uosukainen, M., Kukkonen, S., Karhu, S. 2014. Fruit development in artificially self-and cross-pollinated strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus idaeus*). Acta Agric. Scand. B. 64(5), 408-415.

Vezvaei, A., Jackson, J.F., 1997. Gene flow by pollen in an almond orchard as determined by isozyme analysis of individual kernels and honey bee pollen loads. Acta Hortic. 437. 75–82. https://doi.org/10.17660/ActaHortic.1997.437.6

Waddington, K.D., 1979. Flight patterns of three species of sweat bees (Halictidae) Foraging at Convolvulus, J. Kans. Entomol. Soc. 52 (4). 751-758.

Waddington, K.D., 1980. Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar. Oecologia 44, 199–204. https://doi.org/10.1007/BF00572680

Walters, S.A., Schultheis, J.R., 2009. Directionality of pollinator movements in watermelon plantings. HortScience. 44(1). 49-52

Waser, N.M., 1982. A comparison of distances flown by different visitors to flowers of the same species. Oecologia 55, 251–257. https://doi.org/10.1007/BF00384496

Wietzke, A., Westphal, C., Gras, P., Kraft, M., Pfohl, K., Karlovsky, P., [...], Smit, I. 2018. Insect pollination as a key factor for strawberry physiology and marketable fruit quality. Agric. Ecosyst. Environ. 258, 197-204.

Winfree, R., Williams, N.M., Gaines, H., Ascher, J.S., Kremen, C., 2007. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA, J. Appl. Ecol. 45, 793–802. https://doi.org/10.1111/j.1365-2664.2007.01418.x

Zimmerman, M., 1979. Optimal foraging: A case for random movement. Oecologia. 43, 261–267. https://doi.org/10.1007/BF00344953

Zimmerman, M., 1982. Optimal foraging: random movement by pollen collecting bumblebees. Oecologia 53, 394–398. https://doi.org/10.1007/BF00389020

Connecting text

In Chapter 3 and Chapter 4 I found that small, wild bees provide higher-quality pollination and produce larger strawberries than do honey bees. Foraging behaviour was found to facilitate this effect. Wild bees tended to forage between crop rows more often than honey bees, which increased their potential to transfer cross- or non-clonal pollen. As the previous chapters comprised a detailed investigation of pollination at the flower level, Chapter 5 takes a broader look at the potential strawberry pollinators at the field scale. Specifically, I surveyed wild and managed bees at 12 strawberry fields with two different field-margin habitat types across Eastern Ontario, and assessed the amount of pollen deposited in strawberry flowers at increasing distances from field margins. This chapter is motivated by the questions: 1) How does field-edge habitat type affect the composition of the wild bee community in strawberry fields? and 2) Are small wild bees able to persist in strawberry fields, despite the lack of within-field nesting habitat?

Author contributions: The authors of this work are Gail MacInnis,[1] Chris Buddle,[1] and Jessica Forrest[2]. GM conceived the ideas and designed the study, collected the data, conducted analyses, and wrote the manuscript. JF and CB had input on experimental design, and JF and CB provided critical contributions to the manuscript drafts.

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Chapter 5: Small wild bees decline with distance into strawberry crops regardless of field margin habitat

5.1 Abstract

Flowering crops provide ample floral resources for pollinators, but the time and duration of bloom and a lack of nesting habitat can reduce the suitability of cropland for many wild bee species. The preservation of pollinator habitat on croplands in the form of hedgerows, wildflower strips, and natural and semi-natural areas can help maintain and enhance wild bee populations in agricultural landscapes. However, there have been few comparisons of the effectiveness of different types of field-margin pollinator habitat in maintaining bee diversity and pollination of the focal crops. Here, we compared wild bee abundance, species richness and community composition between strawberry crops bordered by hedgerows, and those bordered by larger expanses of natural land (forests). Strawberry is an important crop in which to investigate pollinator export from field margins as the rows are covered with straw, which reduces habitat for ground-nesting bees within the crop; thus, most wild pollinators would need to enter the crop from the margins. We sampled bees in six strawberry fields with hedgerow margins and six strawberry fields with forested margins; all fields were at least 200 m long. We also examined strawberry pollen deposition at regular intervals into the fields, and the magnitude of pollinator export from the field margins towards the centre of the crops. We found that bees as a group were no more species-rich or abundant in crops bordered by forests than in crops bordered by hedgerows, although large-bodied bees were more abundant in the former than the latter. Regardless of field-margin type, we found that small wild bee abundance declined significantly from the edge to the centre of the crop, but honey bee (*Apis mellifera* L.) and large-bodied bee abundance did not. Strawberry pollen deposition also did not decline with distance into the crop. Although previous work indicates that small wild bees are more effective (yield-increasing) pollinators of strawberry on a per-visit basis, their limited foraging ranges suggest they may only pollinate marginal areas, given typical field sizes in our area.

5.2 Introduction

The movement of organisms from one type of habitat to another (cross-habitat spillover) influences the composition of ecological communities (Ries & Sisk 2004; Hendrickx et al. 2007; Blitzer et al. 2012; Ricketts et al. 2008). As natural lands are increasingly converted to croplands (Lambin & Meyfroidt, 2011), especially pollinator-dependent crops (Aizen & Harder, 2009), cross-habitat spillover becomes vital to the preservation of pollination services provided by wild animals in agricultural landscapes (reviewed by Tscharntke et al. 2012). Wild bees are highly effective pollinators, and can increase the quantity and quality of certain crops relative to managed honey bee pollination (Garibaldi et al. 2013; Klatt et al. 2014). As wild bees are largely unmanaged, they rely heavily on the landscape for floral resources and nesting habitat. Thus, the preservation of non-crop habitat components such as hedgerows, shelterbelts, untilled land, and natural and semi-natural areas can enhance wild bee populations (Kremen et al. 2004; Scheper et al. 2013; Willliams et al. 2015) and pollination services in croplands (Morandin & Winston 2006; Holzschuh et al. 2012; Carvalheiro et al. 2012). Despite the cited importance of cross-habitat spillover, comparisons of pollinator diversity between different non-crop habitat types, and the impact of those habitats on crop pollination, are limited (Chacoff & Aizen 2006; Kohler et al. 2008; Morandin and Kremen 2013). Such comparisons are necessary to determine which types of field margins can maximize spillover from non-crop areas to crop fields and preserve a diversity of wild bees and pollination services in agroecosystems.

Although honey bees can travel several kilometres in search of food (Visscher & Seeley 1982), the foraging ranges of small-bodied bees (e.g., *Hylaeus* spp.) can be less than 150 metres (Gathmann and Tscharntke 2002; Greenleaf et al. 2007; Zurbuchen et al. 2010). Consequently, a lack of nesting habitat (e.g., dead wood, bare ground) in close proximity to crops can reduce the suitability of agroecosystems for many wild bee species, even those that readily forage on flowers of crop plants. In addition to nesting habitat, the availability of a diversity of floral resources in croplands is necessary to support bees with suitable pollen and nectar outside the period of crop bloom. On the other hand, the presence of a diverse floral community on the field margin could have a negative effect on crop pollination, if it results in transfer of heterospecific pollen to crop flowers. Further, if floral resources and nesting habitat are plentiful only at or beyond crop edges, bees (especially small-bodied bees) may prefer to remain within these areas instead of foraging further into the centre of the focal crop (Lander et al. 2011). Thus, the limited foraging range of small-bodied bee species and the availability of floral resources at crop edges has the potential to limit wild bees and pollination to small areas within crop fields (e.g. Kohler et al. 2008).

Strawberry, an economically important crop in Canada (AAFC 2017), is an ideal crop for studying wild bee diversity and cross-habitat spillover, as the area between crop rows is covered with straw, and plastic mulches are increasingly used within crop rows (OMAFRA 2016). This reduces habitat for ground-nesting bees within the crop, forcing most wild pollinators to enter the crop from the margins. This lack of bare ground within strawberry crops offers a natural within-field abundance and richness gradient to examine bee species spillover from natural habitats into cropland. Strawberry yield and quality also increases with insect pollination, and is an attractive pollen and nectar source for wild bee species (Chagnon et al. 1993; Klatt et al. 2014). In this study, we investigated the influence of field-margin type on bee community composition in strawberry fields bordered by forests or hedgerows, the most common field perimeters in the study area. Hedgerows are narrow, linear strips of trees or shrubs, which generally contain fewer native plants than do large natural habitats (Roy and de Blois 2006; Schmucki and de Blois 2009). Given that bee communities in forested areas contrast with those of agricultural areas (Harrison et al. 2017) and forests contain larger expanses of undisturbed areas than hedgerows, we expected that 1) bee community composition would differ between strawberry fields with forested and hedgerow margins, and that species richness and abundance would be less in the latter. With the lack of within-field nesting habitat and the limited foraging ranges of many (especially small) wild bee species, we expected that 2) regardless of margin type, pollinators and pollination would decline with distance from strawberry field edges, with this effect being stronger on small-bodied bees. We also expected that 3) field margins containing abundant and/or diverse flowering plant communities would promote more heterospecific pollen deposition within the crop. By testing all three predictions, we can assess the benefits of different types of field-margins for crop pollination and wild bee communities.

5.3 Methods

5.3.1 Study sites and sampling protocol

This research was conducted at twelve sites in eastern Ontario, Canada, within the Ottawa municipality, primarily east and south of the National Capital Region (45°25’29”N 75°41’42”W). The landscape consisted of a mix of forest, urban, and agricultural areas (Fig. 5.1). Approximately 35% of the area was designated for agriculture, with corn, cereal grains, and hay being the most common field crops (Smith, 2015). However, all sites were located on farms that grew mainly fruit crops, specifically raspberry, strawberry, and apple. The forest patches on farms were part of the Great Lakes–St. Lawrence forest region which is dominated by a mix of hardwoods (*Acer* spp., *Betula* spp.) and coniferous trees (*Tsuga canadensis, Pinus* spp.).

Six of the study fields had at least one margin bordered by a forest, and six fields had at least one margin bordered by a hedgerow. All the fields were planted with June-bearing (short-day), strawberry varieites. Most of the other field margins were bordered by another crop, typically soy or corn in the seedling stage. Each hedgerow site consisted of a strawberry field bordered by a narrow strip of trees (< 20m wide) at the crop edge, whereas forested sites had a patch of contiguous trees at least 200 meters wide. All fields were 200 m to 300 m in length and were at least 1.5 km apart. This distance exceeds the foraging range of most wild bees in our area, except *Bombus* spp., which were excluded from analyses for this reason (21 individuals). We used a matched pairs design with each forest site paired with the closest hedgerow site (Fig. 5.1). To test whether the overall amount of natural habitat differed between site types, we also determined the amount of natural habitat within a 1.2 km radius of each site (including any natural field-edge habitat). This distance corresponds to the maximal foraging range of most bee species in our region, and the amount of natural and semi-natural land at this scale has been positively correlated to wild crop pollinator services (Kremen et al 2004). We used crop inventory maps (AAFC 2016), and image analysis software (ImageJ), to calculate the total area of natural land within the 1.2 km radius. ‘Natural land’ included forested regions (coniferous, broadleaf, mixed wood and undifferentiated), shrublands, wetlands, and grassland meadows. The area of hedgerows and forest patches adjacent to each field site was calculated using ImageJ and Google Earth© satellite images.

All sampling was done on warm (> 18°C), sunny days with little wind (< 2 m/s) over the strawberry bloom period (May 22 – June 6, 2018); paired fields were sampled on the same day. At each field site, 15 m sampling transects within the field and parallel to the field edge were marked at 0 m, 50 m, 100 m and 150 m from the field margin (Fig 5.2A,B). To avoid sampling too close to the opposite field edge, only fields that were 300 m long were sampled out to a distance of 150 m from the field edge (7 fields); those that were only 200 m long (5 fields) were sampled to a distance of only 100 m from the field edge. Timed aerial netting was done at each transect, wherein two observers walked the length of the transects for 15 minutes each (30 minutes total per transect) collecting all wild bees that were seen actively foraging on strawberry flowers. Timing was stopped for each collection event and resumed when the observer was ready to continue searching for bees. All honey bees seen foraging along the transects were counted using a hand-held tally counter but were not collected.

Each site was sampled twice over the blooming period, once in the morning (9h00 to 12h00) and once in the afternoon (13h00 to 15h00). This resulted in a total of 240 minutes of collecting at large (300 m) fields and 180 minutes at small (200 m) fields. All wild bees were identified to species using Ascher & Pickering (2018), two dichotomous keys (Gibbs, 2011; Gibbs et al., 2013), the assistance of an expert in bee taxonomy (see Acknowledgements), and the reference collection at the Biodiversity Centre of the Université de Montréal; voucher specimens are housed at this collection. We also measured the inter-tegular distance (ITD) of each bee species, averaged over 10 individuals (when available). ITD is the distance in millimeters between the two wings and is correlated to body size and foraging range (Greenleaf et al. 2007).

After the final sampling round at each site was complete, we collected 10 flowers in the male phase (*Fragaria* x *ananassa* D. is typically protogynous) at random along each transect for pollen deposition analyses. On each of the flowers collected, the stigmas (10 minimum) at the apex of each receptacle were carefully removed with a scalpel and squashed on a microscope slide with a small cube of fuchsin gel (Dafni & Kevan 2005). We counted the number of strawberry and non-strawberry pollen grains at 40x magnification on the first 10 stigmas encountered under the microscope. The total count of strawberry pollen was divided by 10 to determine the average number of strawberry and non-strawberry pollen grains per stigma at each distance (0 m, 50 m, 100 m, 150 m) from the field margin to the crop centre.

To determine whether floral diversity differed between field-margin types, floral density and richness were also measured at the field margin once at each site, during strawberry bloom. We placed 20, 1m^2 quadrats along and within the hedgerow or forest at approximately 5 m intervals, parallel to the field edge, and counted all open flowers inside the quadrats. Ten quadrats were placed at the edge of the forest or hedgerow just beyond the tree line, and 10 quadrats were placed at 5 m inside the hedgerow or forest. For plants with many small, compact flowers such as *Cornus alternifolia*, each inflorescence was counted as one flowering unit (as in Fründ et al. 2010). All flowering plants were identified to species using Peterson and McKenny (1996). We also measured the abundance of all open strawberry flowers within the crops using 15, 1 m^2 quadrats placed along the length of the 15 m transects.

5.3.2 Statistical Analyses

Field margin habitat and the crop bee community

Samples were pooled across transects and sampling periods and the total abundance and diversity metrics were calculated for the bee communities of each site. The bee communities at fields with forested and hedgerow margins were compared using non-metric multidimensional scaling, with Bray-Curtis distances (NMDS, package: vegan, Oksanen et al. 2019). A four-axis solution was used as it lowered the final stress below 0.10 and additional axes resulted in little improvement. To compare species richness for a given number of specimens, taxon sampling curves (rarefaction) were generated for all sites (package: vegan). We constructed generalized linear mixed-effect models (GLMMs, package: lme4, Bates et al. 2015) to compare wild bee abundance and species richness between field-margin types. For each model, field-margin type was a fixed effect, and pair ID was a random effect. To account for overdispersion, a negative binomial distribution was used for the abundance models. A Gaussian distribution was used for species richness models with the rarified number of species as the response variable (abundance differed across sites despite consistency in sampling effort). Comparisons of bee diversity (Shannon index) between field-margin types were done in the same manner using LMMs. Field-margin flower data were pooled across quadrats at each site and we compared floral abundance and diversity between margin types with paired t-tests.

To further examine the responses of individual bee species to each field-margin type, we modeled changes in the relative abundance of the 20 most abundant bee species using multinomial models. The models were fitted using the package ‘mvabund’ (Yang et al. 2019), with the function ‘manyglm’. This function fits a separate generalized linear mixed model to each species in the abundance matrix. The abundance of each species was the response variable and field-margin type and distance were fixed effects; pair ID was included as a random effect in all models. We calculated the influence of predictor variables on individual species using likelihood-ratio tests.

Pollinator and pollen export

To examine the influence of distance into the crop and field-margin type on wild bee abundance, we constructed negative binomial GLMMs with distance into the field (continuous variable) and the interaction between distance and field-margin type as fixed effects, and pair ID as a random effect. In addition, we tested whether bee spatial distributions through the fields were affected by bee body size with a GLMM. Bee abundance was the response variable and distance and body size (average ITD in mm for each species) were fixed predictors; Pair ID was a random effect. We also constructed Gaussian GLMMs to test for differences in strawberry flower abundance and strawberry pollen deposition between field-margin types. Field-margin type and distance from the field edge (and the interaction between the two) were fixed effects and pair ID was a random effect. For the pollen models, the average number of strawberry pollen grains per stigma was the response variable. To examine heterospecific pollen deposition with distance into the field, we used a zero-inflated negative binomial GLMM, package: pscl (Zeileis et al. 2008). The average number of heterospecific pollen grains per stigma was the response variable, distance from the field edge was a fixed numerical predictor and pair ID was a random effect.

5.4 Results

In total, we collected 784 individual wild bees comprising 70 species in 15 genera, and observed 1393 honey bee individuals foraging on strawberry flowers (see Table S5.1 for full species list). Only seven male bees were collected in total and these were not included in analyses, as they do not collect pollen or depend on nesting habitat. Likewise, the five parasitic individuals found (*Nomada* spp., *Sphecodes* spp., Table S5.1) were not included in analyses since their presence is more dependent on host species than habitat characteristics (Williams et al. 2010). Honey bees were not included in diversity or body size analyses.

Field edges contained 18 species of plants that were in flower during sampling. Hedgerow margins contained five flowering shrubs and two tree species. Forested margins contained four tree species and two shrubs. Both field-margin types included the same six flowering herbaceous species (see Table S5.2 for full species list). The average number of flowering plant species in the field margin did not differ significantly between forested and hedgerow margins, nor did floral abundance (Table 5.1). Strawberry flower abundance did not change with field-margin type (Table 1) or with distance into the field ($\chi^2_1 = 1.03$, $p = 0.31$). There was more natural land at forested fields than hedgerow fields within a 1.2 km radius (Table 5.1). The area of natural land directly adjacent to the fields was correlated with the amount of natural land within a 1.2 km radius of the fields ($r_p = 0.51$).

5.5.1 Field margin habitat and the crop bee community

Overall wild bee abundance did not differ between field-margin types among the sites examined (Table 5.1). Similarly, species richness and Shannon diversity did not differ between fields bordered by forests and fields bordered by hedgerows (Table 5.1).

The ordination plot indicated that the composition of the bee communities did not differ between field-margin types, as there was complete overlap between ellipses (Fig. 5.3A). The hedgerow rarefaction curves were of similar shape to those of the forested sites, with the exception of two forested sites that were composed of fewer, more abundant species (Fig 5.3B). Of the 20 most abundant bee species, only three differed significantly in abundance between field-margin types: There were more *Agapostemon sericeus, Andrena carlini*, and

Augochlorella aurata individuals at forested sites (Table S5.3). When categorized by size (large bees = ITD > 2 mm, small bees ≤ 2 mm), there were more large bees at forested than hedgerow sites, but small bee abundance was not affected by field-margin type (Table 5.1).

5.5.2 Pollinator and pollen export

Honey bee abundance did not decline significantly with distance into the crop (Table 5.2, Fig. 5.4A). The abundance of wild bees decreased with distance consistently across field-margin types (Table 5.2; Fig. 5.3B); there was no interaction between field-margin type and distance (χ^2_1 = 0.19, *p* = 0.66). This decline was driven by a decrease in the number of small bees, as ITD increased significantly with distance towards the crop centre (β = 0.12, *t* = 2.48, *p* = 0.013, Fig. 5.4C, Table 5.2). This general decline of small bees with distance was also observed in the analyses of the most abundant species, with 6 of 8 small-bodied species declining with distance (Table S5.3). At 150 m into the field, the number of small bees was 44% less than at the field edge. The abundance of large wild bees did not differ with distance into the crop (Table 5.2) and the influence of distance on large bees did not change with field-margin type (field-margin type x distance: χ^2_1= 0.93, *p* = 0.33).

The amount of heterospecific pollen deposited within strawberry flowers was negligible, and was not significantly affected by field-margin type (χ^2_1 = 0.73, *p* = 0.39) or distance into the field (Table 5.2). This suggests that bees foraging in the field margins did not transport a significant amount of non-strawberry pollen into the crop. Strawberry pollen deposition was not affected by distance from the field edge (Table 5.2, Fig. 4D).

5.5 Discussion

Wild bees are effective crop pollinators that rely heavily on the habitat and floral resources in croplands and surrounding natural and semi-natural areas. Thus, the preservation of natural elements of agricultural landscape can preserve their pollination services in crops (Garibaldi et al. 2011). Contrary to our expectations that forested field margins would increase the richness and abundance of the crop bee communities throughout strawberry crops, fields surrounded by larger expanses of natural areas did not have more abundant or species-rich wild bee communities than fields bordered by narrow strips of natural land (hedgerows). This unexpected result may be due to the composition of the larger landscape. When crops are grown within a mix of natural and uncultivated land, bee diversity and crop productivity are higher than in monocultures or simplified landscapes (Hendrickx et al. 2007; Ricketts et al. 2008; Carvalheiro et al. 2011). Habitat options like hedgerows may not influence pollinator populations above the background influence of heterogeneity in complex landscapes, as they would in more simplified landscapes (Tscharntke et al., 2005, Motzke et al. 2016). The landscapes surrounding our study sites would be considered complex as they contained a mosaic of forested, urban, and agricultural lands, with many small habitat islands (Fig 5.1). The heterogeneity of the landscapes at the regional scale may explain why we did not find a difference in bee communities between field-margin types at the local scale. Further, forests may not provide the ideal habitat for bees (Mandelik et al. 2012). Hedgerow areas are more open, and may provide more warmth and floral resources throughout the season than forest interiors. It is more likely that we would have found dissimilar bee communities had we compared fields with hedgerows to fields with bare margins (e.g. Moradin et al. 2013).

Bee abundance and species richness have been positively correlated with the abundance and richness of flowering plant species (Steffan-Dewenter & Tscharntke 2001; Potts et al. 2003; Sutter et al. 2017). Although we did not measure floral resources deep within the forest, the diversity of floral resources near the field margin did not differ between forested and hedgerow sites. As June-bearing strawberries bloom early in the season, bee species richness might have increased at our study sites as more flowering plants and bees emerged, and differences between field-margin types might have appeared, if, for example, forests support more late-season than early-season bees. However, this study was focused on wild pollinator services to strawberry crops, so plant or bee species that emerged after our sampling period (which encompassed the entire strawberry bloom) would not have influenced strawberry pollination.

Most bee species found at our sites were also regionally common species. Wild bee communities in agroecosystems often consist of common species that are well-adapted to live near crops, and can persist despite declines in surrounding natural areas or flowering plant diversity (Kleijn et al. 2015). For example, the two *Ceratina* species found in this study area (*C. dupla* and *C. calcarata*) were most abundant on farms growing raspberry, likely because raspberry (*Rubus* spp.) stems are a preferred nesting substrate (Vickruck et al. 2011; Vickruck & Richards 2012). This suggests that the presence of raspberry matters more for *Ceratina* abundance than the amount of natural habitat or richness of floral resources surrounding the farm.

Although the overall abundance of wild bees was unaffected by field-margin type, the abundance of large bees was higher in fields surrounded by forests rather than hedgerows. Studies of other systems have found that populations of large-bodied bees are affected by the composition of the landscape at larger spatial scales than small-bodied bees (Benjamin et al. 2013, Aguirre-Gutiérrez et al. 2015; Warzecha et al. 2016). The greater foraging ranges of larger-bodied bees mean they can access resources further from their nests and interact with the landscape at large spatial scales (Cresswell et al. 2000; Gathmann & Tscharntke 2002; Greenleaf et al. 2007). However, forest-margin fields also had more overall natural habitat in our study, so the effects of field-margin type on large-bodied bees cannot be separated from effects of broader landscape composition. The positive relationship between body size and distance from the field margin (Figure 5.3B) suggests that foraging range and the lack of within-field habitat limit the abundance of small wild bees in field interiors. If small wild bees are better pollinators of strawberry (as in MacInnis & Forrest 2019) than large-bodied bees, the lower abundance of small bees within the crop could reduce the quality of pollination services to strawberry fields in this region. While research suggests that small bees (ITD < 2 mm) should have foraging ranges of 200 m to 500 m (Greenleaf et al. 2007), only 13% of bees at this size range were found beyond 100 m of the field margin in our study. The larger bee species (e.g. *Halictus rubicundus, Agapostemon* spp., *Andrena* (*Melandrena*) spp., Table S1) were unaffected by distance from the field margin and may be able to provide pollination services further into strawberry fields (Fig. 5.4C). However, the number of honey bees observed visiting flowers in field interiors was more than double that of large wild bees. This suggested that honey bees were most likely providing more pollination than wild bees in the field interiors.

The low number of wild bees found within the crop area provides support to our second hypothesis, that the benefit of crop enhancements to agricultural production may be limited to small areas within the crop, regardless of field-margin type. The density of strawberry flowers was 37 flowers/m^2; as each flower needs as least four visits for pollination, this amounts to 2220 bee visits needed along the 15 m transects (assuming each observer was able to see half a meter in each direction while traversing the transects). On average, 33 wild bees were observed visiting strawberry flowers along the transects per site per day. If we assume bees are active for about 8 hours per day, we captured 6.25% of the foraging bees in our 30 minute sampling periods. This amounts to 528 bee visits each day along the transects, as strawberry flowers are receptive for 2-3 days, this is below the number of visits required for full pollination.

It has been suggested that within a 1.2-1.5 km radius, approximately 30% of land area should be devoted to natural or semi-natural habitat to maintain wild pollinator services to crops with high pollinator dependency (watermelon: Kremen *et al*., 2004). The average non-crop land area here was 23% across sites, and although strawberry requires fewer bee visits for full pollen deposition than watermelon, this amount of land is likely not high enough to provide enough wild pollinator habitat and services throughout large strawberry crops. Since pollen deposition did not decline with distance into the field, and the number of honey bees was double that of wild bees beyond the field edge, it is reasonable to assume that honey bees were responsible for pollinating more strawberry plants than wild bees at the field scale.

Implications

Spillover from natural or semi-natural habitats into agroecosystems can be beneficial for pest control and pollination services (Tscharntke et al. 2012). The provision of pollinator habitat is not a requirement on Canadian farms (AAFC, 2014), and the choice to preserve or remove potential pollinator habitat belongs to the growers. In the case of strawberry, pollination by wild, especially small, bee species has been shown to increase strawberry size (MacInnis & Forrest 2019). Consequently, increases in yield may justify the costs of taking a portion of land out of production for pollinator habitat, as strawberry crops under conventional management are essentially devoid of within-field habitat options for ground-nesting bees. Here, foraging range and a lack of within-crop nesting habitat limited small wild bee abundance within the crop. Providing habitat options (flower strips, bare ground) within the crop as well as accessible floral resources outside of the crop bloom period may increase wild bee abundance within the crop, but this possibility was not tested here. We found a negligible amount of heterospecific pollen transfer from edge plants, even to strawberry plants immediately adjacent to flowering weeds at the field margin (Table 2). Thus, incorporating flower strips within the crop should not negatively affect strawberry pollination, although it may impede machinery and agrochemical applications.

Narrow strips of undisturbed land, flowering patches, or habitat fragments may be effective in increasing wild bee abundance on strawberry farms. Comparative assessments of these strategies can increase our understanding of how species differ in their edge responses, and the amount of habitat needed to sustain wild pollinator services in croplands. It is also important to note that simple measures of species richness and abundance cannot detect differences in the species composition of bee communities or the effects of landscape characteristics on individual species or groups (e.g. large and small bees). Measuring species-specific responses to habitat size, configuration, land cover types, and the quality of natural habitat remnants is vital to preserve and enhance bee diversity and ecosystem services in human-dominated landscapes, and to reduce dependency on managed honey bees.

Table 5.1. Average bee and floral diversity in strawberry fields bordered by forests and hedgerows and the results of the comparisons of each diversity metric. Bee abundance was based on a total of 240 minutes of collecting at large (300 m) fields and 180 minutes at small (200 m) fields. The rarefied number of species was based on 40 individuals, the lowest number of individuals found at any site. N = 6 each for forested and hedgerow sites. χ^2 indicates the Wald chi-squared test statistic, and t indicates a t-test was used for comparisons. p is the significance.

Response variable	Forest (mean ± SD)	Hedge (mean ± SD)	Test statistic	*p*
Wild bee abundance (no. of individuals)	19.7 ± 12.9	16.6 ± 8.5	1.02 (χ^2)	0.30
Honey bee abundance (no. of individuals)	39.5 ± 24.4	25.6 ± 15.3	3.29 (χ^2)	0.67
Bee species richness (no. of species)	10.9 ± 4.9	9.5 ± 3.7	0.21 (χ^2)	0.65
Large bee abundance (no. of individuals)	27.5 ± 13.2	19.8 ± 12.3	7.42 (χ^2)	**0.006**
Small bee abundance (no. of individuals)	41.3 ± 19.3	42.0 ± 17.7	0.03 (χ^2)	0.86
Bee diversity (Shannon index)	2.1 ± 0.6	2.3 ± 0.2	0.77 (χ^2)	0.38
Edge floral richness (no. of species)	3.8 ± 1.3	4.3 ± 1.2	0.74 (*t*)	0.49
Edge floral abundance (floral units/m^2)	42.3 ± 46.5	46.7 ± 32.6	0.09 (*t*)	0.93
Strawberry flower abundance (floral units/m^2)	41.1 ± 37.3	33.1 ± 28.8	0.79 (*t*)	0.47
Natural land-1.2 km radius (km^2)	1.34 ± 0.34	0.81 ± 0.30	8.18 (χ^2)	**0.004**

Table 5.2. Summary of bee diversity and pollen deposition as functions of distance from field margins in strawberry fields bordered by hedgerows and forests (N=12). Large bees had an inter-tegular distance of greater than 2 mm, small bees had an inter-tegular distance of less than or equal to 2 mm. χ^2 is the value of the Wald chi-squared test statistic. p is the significance value. Significant relationships are indicated in bold.

Distance	0 m (mean ± SD)	50 m (mean ± SD)	100 m (mean ± SD)	150 m (mean ± SD)	χ^2	*p*
Wild bee abundance (no. of individuals)	25.4 ± 10.0	16.2 ± 8.6	14.6 ± 10.0	14.9 ± 13.1	4.89	**0.027**
Honey bee abundance (no. of individuals)	36.6 ± 15.9	31.0 ± 18.8	31.2 ± 26.5	29.6 ± 26.6	1.99	0.16
Species richness (no. of species)	12.7 ± 4.2	10.4 ± 4.0	8.8 ± 4.2	7.9 ± 3.8	11.38	**<0.001**
Large bee abundance (no. of individuals)	21.1 ± 14.3	23.2 ± 15.0	23.2 ± 20.4	21.7 ± 19.8	0.27	0.61
Small bee abundance (no. of individuals)	14.1 ± 9.3	7.7 ± 5.5	7.8 ± 7.7	7.9 ± 7.6	8.28	**0.004**
Strawberry pollen (grains per stigma)	15.8 ± 8.0	14.6 ± 5.8	13.7 ± 6.0	14.0 ± 6.8	0.24	0.62
Heterospecific pollen (grains per stigma)	0.4 ± 0.4	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.3	3.00	0.09

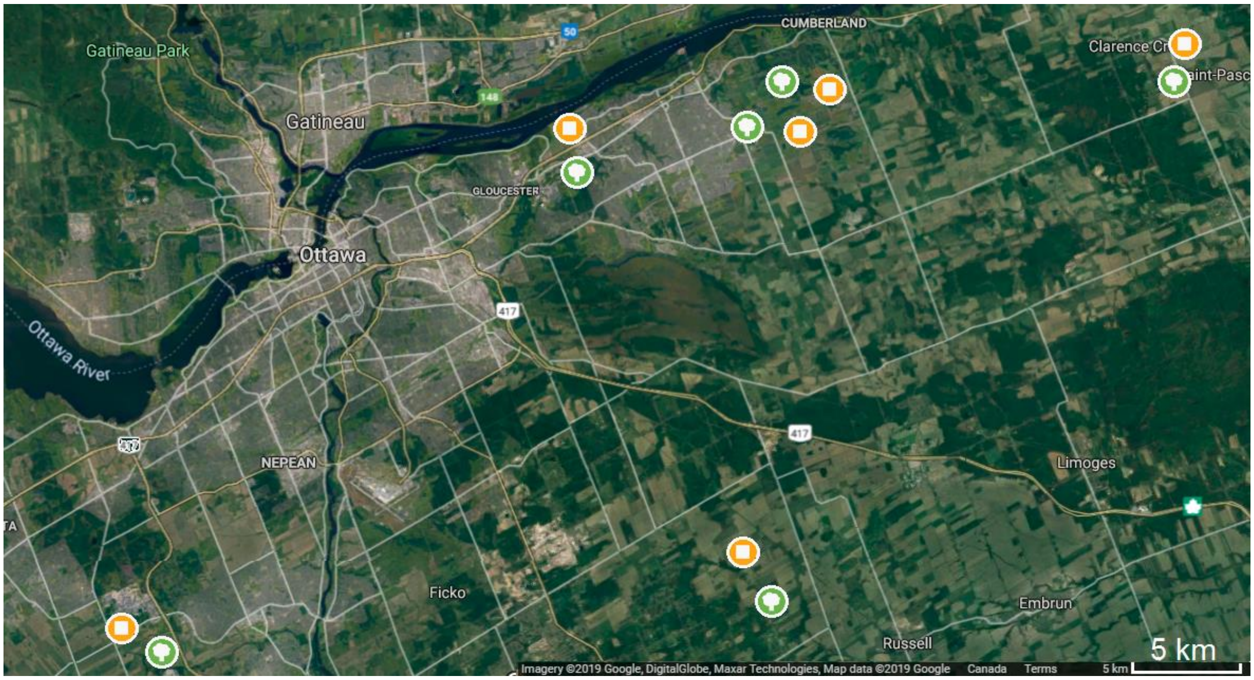

Figure 5.1. Map of strawberry fields bordered by forests (green trees) and hedgerows (orange squares) in the region of Ottawa, Ontario. Six forested sites were matched with six hedgerow sites.

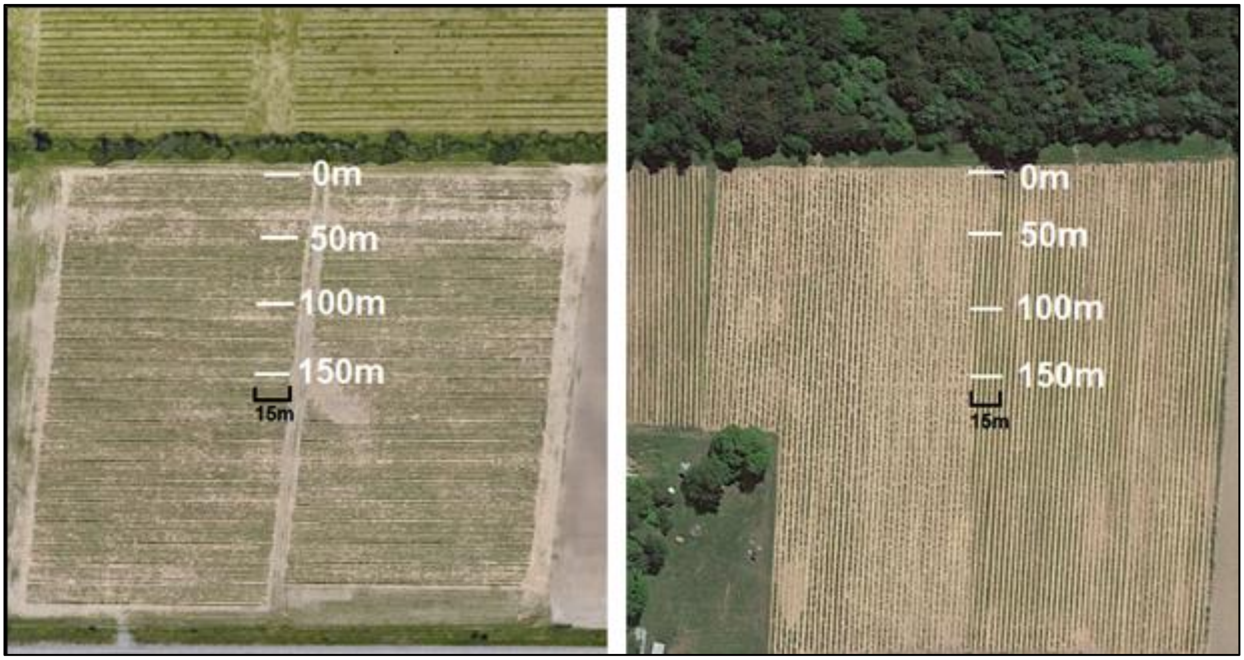

Figure 5.2. Sampling design and field-margin types. A) Strawberry field with hedgerow margin and B) strawberry field with forested margin. Sampling transects and distances from the field margin are marked in white.

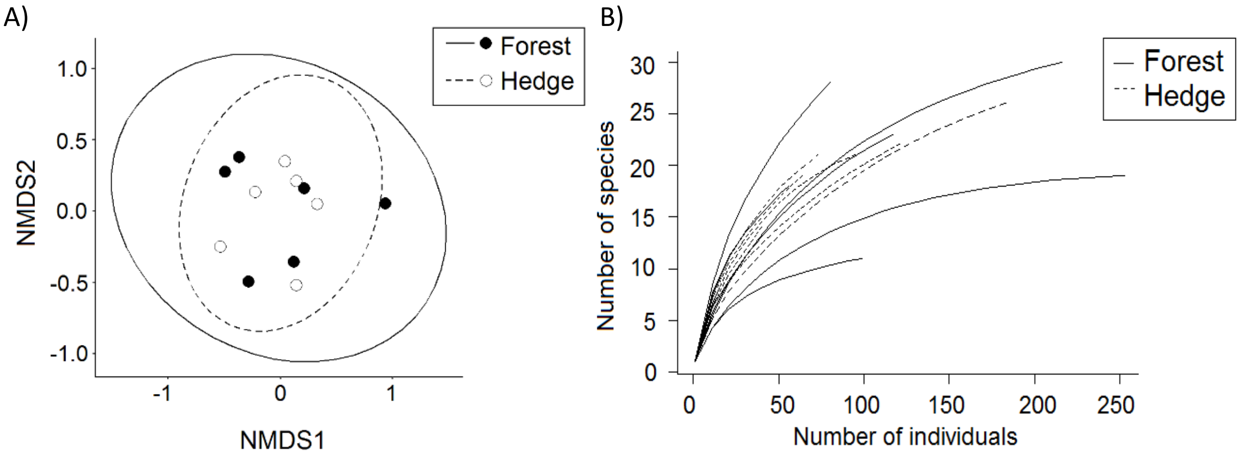

Figure 5.3. Bee community composition at strawberry fields with forested and hedgerow field margins. A) Non-metric multidimensional scaling of the 12 sites based on species abundance (Bray-Curtis dissimilarities). Hedgerow sites are represented by open circles and the dotted ellipse. Forested sites are filled circles surrounded by a solid ellipse. B) Rarefaction curves for the 12 sites; forested sites are solid lines, hedgerow sites are dotted lines.

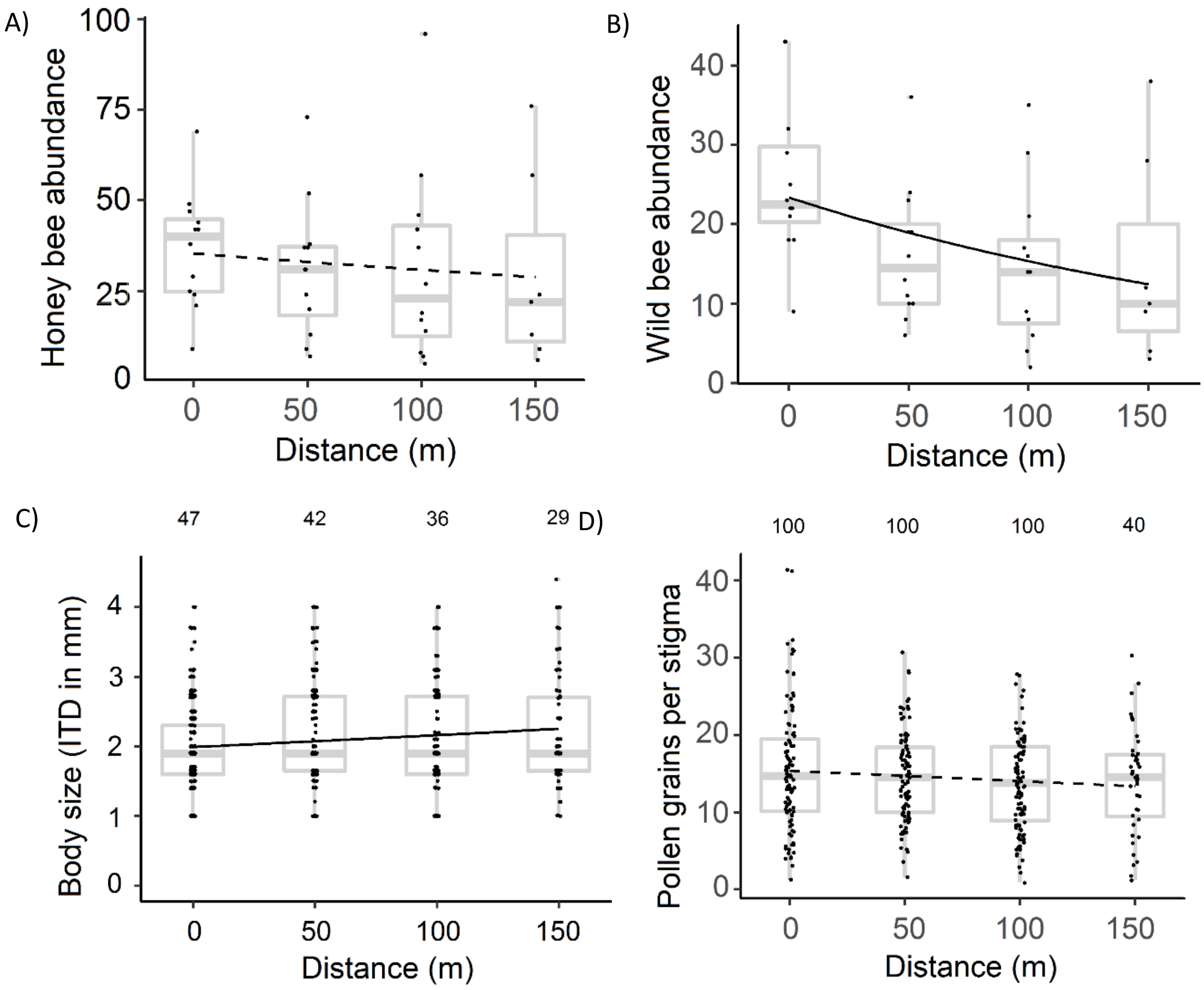

Figure 5.4. A) Honey bee abundance with distance from the field edge. Points represent the number of honey bees found foraging at each distance from the field margin at each of the 12 sites. B) Wild bee abundance with distance from the field edge. Points represent the number of wild bee individuals found foraging on strawberry flowers at each of the 12 sites. C) Body size of wild bees with distance from the field edge. Points are the ITD values (the distance between the tegula of each bee) for each bee species at each distance from the field edge over the 12 sites. D) The number of strawberry pollen grains per stigma at the field edge inward to 150 m for six fields, and to 100 m for four of the fields. Sample size is listed above. All grey boxes show the interquartile range, the median is indicated by a horizontal line, and whiskers indicate the data range. Solid lines are the regressions from the generalized linear mixed models. Dotted lines are non-significant regression lines. All points are jittered horizontally for clarity.

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5.7 References

Agriculture and Agrifood Canada (AAFC), 2017. Crop Profile for Strawberry in Canada. Accessed February 2019. http://publications.gc.ca/collections/collection_2019/aac-aafc/A118-10-17-2016-eng.pdf

Agriculture and Agrifood Canada (AAFC), 2016. Annual Crop Inventory, Interactive Maps. Accessed April 2019. http://www.agr.gc.ca/atlas/aci/

Agriculture and Agrifood Canada (AAFC), 2014. Native Pollinators and Agriculture in Canada. Accessed February 2019. http://publications.gc.ca/collections/collection_2014/aac-aafc/A59-12-2014-eng.pdf

Aizen, M. A., & Harder, L. D., 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Current biology 19(11), 915-918.

Ascher J.S., Pickering J., 2019. Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Retrieved from http://www.discoverlife.org/mp/20q?guide=Apoidea_species

Aguirre-Gutiérrez, J., Biesmeijer, J.C., van Loon, E.E., Reemer, M., WallisDeVries, M.F., Carvalheiro, L.G., 2015. Susceptibility of pollinators to ongoing landscape changes depends on landscape history. Divers. Distrib. 21(10), 1129–1140.

Bailey, S., Requier, F., Nusillard, B., Roberts, S.P.M., Potts, S.G., Bouget, C., 2014. Distance from forest edge affects bee pollinators in oilseed rape fields. Ecol. Evol. 4(4), 370–380.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67(1), 1-48.

Benjamin, F.E., Reilly, J.R., Winfree, R., 2013. Pollinator body size mediates the scale at which land use drives crop pollination services. J. Appl. Ecol. 51(2), 440–449.

Blitzer, E.J., Dormann, C.F., Holzschuh, A., Klein, A.-M., Rand, T.A., Tscharntke, T., 2012. Spillover of functionally important organisms between managed and natural habitats. Agric. Ecosyst. Environ. 146(1), 34–43.

Carvalheiro, L.G., Veldtman, R., Shenkute, A.G., Tesfay, G.B., Pirk, C.W.W., Donaldson, J.S., Nicolson, S.W., 2011. Natural and within-farmland biodiversity enhances crop productivity. Ecol. Lett. 14(3), 251–259.

Carvalheiro, L.G., Seymour, C.L., Nicolson, S.W., Veldtman, R., 2012. Creating patches of native flowers facilitates crop pollination in large agricultural fields: mango as a case study. J. Appl. Ecol. 49(6), 1373–1383.

Chacoff, N.P., Aizen, M.A., 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. J. Appl. Ecol. 43(1), 18–27.

Chagnon, M., Gingras, J., DeOliveira, D., 1993. Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). J. Econ. Entomol. 86(2), 416–420.

Cresswell, J.E., Osborne, J.L., Goulson, D., 2000. An economic model of the limits to foraging range in central place foragers with numerical solutions for bumblebees. Ecol. Entomol. 25(3), 249–255.

Dafni, A., Kevan, P. G., & Husband, B. C. (2005). *Practical pollination biology.* Enviroquest Ltd.

Fründ, J., Linsenmair, K.E., Blüthgen, N., 2010. Pollinator diversity and specialization in relation to flower diversity. Oikos 119(10), 1581–1590.

Garibaldi, L. A., Steffan-Dewenter, I., Kremen, C., Morales, J. M., Bommarco, R., Cunningham, S. A., [...] & Holzschuh, A., 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecol. Lett. 14(10), 1062-1072.

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., [...] & Bartomeus, I., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127), 1608-1611.

Gathmann, A., Tscharntke, T., 2002. Foraging ranges of solitary bees. J. Anim. Ecol. 71(5), 757–764.

Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia 153(3), 589–596.

Gibbs, J., 2011. Revision of the metallic *Lasioglossum* (Dialictus) of eastern North America (Hymenoptera: Halictidae: Halictini). Zootaxa, 3073(1), 1-216.

Gibbs, J., Packer, L., Dumesh, S., & Danforth, B.N., 2013. Revision and reclassification of *Lasioglossum* (Evylaeus), L.(Hemihalictus) and L.(Sphecodogastra) in eastern North America (Hymenoptera: Apoidea: Halictidae). Zootaxa, 3672, 1-117.

Greenleaf, S.S., Williams, N.M., Winfree, R., & Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia 153(3), 589-596.

Hendrickx, F., Maelfait, J.-P., Van Wingerden, W., Schweiger, O., Speelmans, M., Aviron, S., Augenstein, I., […], Bugter, R., 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. J. Appl. Ecol. 44(2), 340–351.

Holzschuh, A., Dudenhöffer, J.-H., Tscharntke, T., 2012. Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biol. Conserv. 153, 101–107.

James, J., 2011. *Native Bee Diversity in Conventional and Organic Hedgerows in Eastern Ontario.* Doctoral dissertation, Carleton University.

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. 2014. Bee pollination improves crop quality, shelf life and commercial value. Proc. R. Soc. Lond., B, Biol. Sci., 281(1775), 20132440.

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.-M., […], Potts, S.G., 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 7414.

Kohler, F., Verhulst, J., Klink, R. Van, Kleijn, D., 2008. At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes? J. Appl. Ecol. 45(3), 753–762.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., [...], Ricketts, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol. Lett. 10(4), 299–314.

Kremen, C., Williams, N.M., Bugg, R.L., Fay, J.P., Thorp, R.W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecol. Lett. 7(11), 1109–1119.

Lambin, E.F., Meyfroidt, P., 2011. Global land use change, economic globalization, and the looming land scarcity. Proc. Natl. Acad. Sci. U.S.A. 108(9), 3465–72.

Lander, T.A., Bebber, D.P., Choy, C.T.L., Harris, S.A., Boshier, D.H., 2011. The Circe principle explains how resource-rich land can waylay pollinators in fragmented landscapes. Curr. Biol. 21(15), 1302–1307. https://doi.org/10.1016/J.CUB.2011.06.045

MacInnis, G., Forrest, J.R.K., 2019. Pollination by wild bees yields larger strawberries than pollination by honey bees. Journal of Applied Ecology, 56(4), 824-832.

Morales, C.L., Traveset, A., 2009. A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecol. Lett. 12(7), 716–728.

Morandin, L.A., Kremen, C., 2013. Hedgerow restoration promotes pollinator populations and exports native bees to adjacent fields. Ecol. Appl. 23(4), 829–839.

Morandin, L.A., Winston, M.L., 2006. Pollinators provide economic incentive to preserve natural land in agroecosystems. Agric. Ecosyst. Environ. 116(3-4), 289–292.

Motzke, I., Klein, A.-M., Saleh, S., Wanger, T.C., Tscharntke, T., 2016. Habitat management on multiple spatial scales can enhance bee pollination and crop yield in tropical homegardens. Agric. Ecosyst. Environ. 223, 144–151.

Oksanen, J.F, Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, […], Wagner, H., 2019. vegan: Community Ecology Package. R package version 2.5-4.

The Ontario ministry of Agriculture, Food and Rural Affaris (OMAFRA), 2016. Dayneutral Strawberries. Production Factsheet. Accessed April 2019: http://www.omafra.gov.on.ca/english/crops/facts/89-099.htm

Peterson, R. T., McKenny, M., 1996. *A field guide to wildflowers: northeastern and north-central North America*. Houghton Mifflin Harcourt. U.S.A.

Potts, S.G., Vulliamy, B., Dafni, A., Ne'eman, G., Willmer, P., 2003. Linking bees and flowers: how do floral communities structure pollinator communities? Ecology 84(10), 2628–2642.

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P., Howlett, B. G., Winfree, R., [...], Bommarco, R., 2016. Non-bee insects are important contributors to global crop pollination. Proc. Natl. Acad. Sci. U.S.A., 113(1), 146-151.

Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., […], Viana, B.F., 2008. Landscape effects on crop pollination services: are there general patterns? Ecol. Lett. 11(5), 499–515.

Ries, L., Sisk, T.D., 2004. A predictive model of edge effects. Ecology 85(11), 2917–2926.

Roy, V., Blois, S. de, 2006. Using functional traits to assess the role of hedgerow corridors as environmental filters for forest herbs. Biol. Conserv. 130(4), 592–603.

Schmucki, R., de Blois, S., 2009. Pollination and reproduction of a self-incompatible forest herb in hedgerow corridors and forest patches. Oecologia 160(4), 721–733.

Scheper, J., Holzschuh, A., Kuussaari, M., Potts, S.G., Rundlöf, M., Smith, H.G., Kleijn, D., 2013. Environmental factors driving the effectiveness of European agri-environmental measures in mitigating pollinator loss - a meta-analysis. Ecol. Lett. 16(7), 912–920.

Smith, P. G., 2015. Long-term temporal trends in agri-environment and agricultural land use in Ontario, Canada: transformation, transition and significance. Journal of Geography and Geology, *7*(2), 32.

Steffan-Dewenter, I., Tscharntke, T., 2001. Succession of bee communities on fallows. Ecography 24(1), 83–93.

Sutter, L., Jeanneret, P., Bartual, A.M., Bocci, G., Albrecht, M., 2017. Enhancing plant diversity in agricultural landscapes promotes both rare bees and dominant crop-pollinating bees through complementary increase in key floral resources. J. Appl. Ecol. 54(6), 1856–1864.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity agroecosystem service management. Ecol. Lett. 8(8), 857–874.

Tscharntke, T., Tylianakis, J.M., Rand, T.A., Didham, R.K., Fahrig, L., Batáry, P., Bengtsson, J., […], Westphal, C., 2012. Landscape moderation of biodiversity patterns and processes - eight hypotheses. Biol. Rev. 87(3), 661–685.

Vickruck, J.L., Rehan, S.M., Sheffield, C.S., Richards, M.H., 2011. Nesting biology and DNA barcode analysis of *Ceratina dupla* and *C. mikmaqi*, and comparisons with *C. calcarata* (Hymenoptera: Apidae: Xylocopinae). Can. Entomol. 143(3), 254–262.

Vickruck, J.L., Richards, M.H., 2012. Niche partitioning based on nest site selection in the small carpenter bees *Ceratina mikmaqi* and *C. calcarata*. Anim. Behav. 83(4), 1083–1089.

Visscher, P. K., & Seeley, T. D., 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology, 63(6), 1790-1801.

Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., Warton, D., 2019. mvabund: statistical methods for analysing multivariate abundance data. R package version 4.0.1. https://CRAN.R-project.org/package=mvabund

Warzecha, D., Diekötter, T., Wolters, V., Jauker, F., 2016. Intraspecific body size increases with habitat fragmentation in wild bee pollinators. Landsc. Ecol. 31(7), 1449–1455.

Williams, N.M., Crone, E.E., Roulston, T.H., Minckley, R.L., Packer, L., Potts, S.G., 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biol. Conserv. 143(10), 2280–2291.

Williams, N.M., Ward, K.L., Pope, N., Isaacs, R., Wilson, J., May, E.A., Ellis, J., Daniels, J., Pence, A., Ullmann, K., Peters, J., 2015. Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. Ecol. Appl. 25(8), 2119–2131.

Zeileis, A., Kleiber, C., Jackman, S. 2008. Regression models for count data in R. J. Stat. Softw. 27(8). http://www.jstatsoft.org/v27/i08/.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., Dorn, S., 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Biol. Conserv. 143(3), 669–676.

Chapter 6: Discussion and Conclusions

Investigations of biodiversity and ecosystem services require detailed study of the mechanisms driving the relationship between the two, and of the external influences on the communities of organisms driving ecosystem functions. The goal of this research was to explore both of these connections through interactions between crop plants and their pollinators. In this chapter, I summarize my results and discuss the broader implications and limitations of my work, with particular reference to ecosystem services and crop pollination. I conclude with a discussion of specific recommendations for managing pollination in strawberry agroecosystems and further research opportunities.

6.1 Bee diversity and crop pollination services

The benefits humans gain from ecosystems (ecosystem services) have become a central rationale for biodiversity conservation, and have been integrated into many environmental assessments and policy recommendations. This includes the Millennium Ecosystem Assessment (2005), the Aichi Targets of the Convention on Biodiversity (2010) and the Global Assessment Report on Biodiversity and Ecosystem Services (2019) by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES). Pollination is an ecosystem service that has received much attention, especially crop pollination. Indeed, the IPBES also produced a large report specifically on pollinators, pollination, and food production (2016). This report points out the lack of research surrounding the role of pollinator diversity and the relative importance of wild versus managed pollinators for crop production (pg. 727). However, the global assessments emphasize conservation of pollinator diversity for the provision of pollination services. These larger claims surrounding pollinator diversity and ecosystem services are yet to be substantiated, as more research is required to assess how changes in the diversity and composition of pollinator communities affect pollination and food production.

The main goal my research was to shed more light on the relationship between pollinator community composition and crop pollination. The development of the method of pollen deposition measurement in Chapter 2 allowed a thorough examination of the pollen deposition by multiple flower visitors in the field, and direct analyses of fruit development. This is the first technique to enable such detailed pollination measurements, and does not rely on traditional single-visit measures of pollinator performance (summarized in Ne'eman et al. 2010). Using this technique, I found that species richness in wild bee pollinators of strawberry did not affect pollen deposition or fruit size (Chapter 3, Fig. 3.1). Previous studies have found species richness in pollinator communities to increase fruit and seed yields (Hoehn et al. 2008, Fründ et al. 2013; Mallinger & Gratton 2015). These studies are based on indirect measures of pollination or artificially constructed bee communities, which may account for the contradictory results. In my study, all wild bee visitors consisted of those foraging naturally within the crop (although the order and type of bees to visit the flowers were controlled), and the pollen contribution was measured by each bee directly. Further, the results of my work are limited to the year and site in which my studies were conducted. This may partly explain why my results differ from other studies that have found positive effects of pollinator diversity on crop yield in strawberry and other cropping systems (Chagnon et al. 1993; Hoehn et al. 2008; Martins et al. 2015; Cusser et al. 2016). However, these, and other studies, of crop pollination are regularly conducted over one or two seasons. It also important to note that the composition of bee communities can vary drastically over time, and my work cannot address whether species rich communities enhance ecosystem stability or resilience to disturbance over larger timescales (e.g. Winfree et al. 2008; Rogers et al. 2014).

The use of economic ecosystem service incentives such as yield increases due to pollinator diversity can motivate conservation actions (e.g. Garibaldi et al. 2016), but this rationale is not without risk. When profiting from ecosystem services is a central motivation for biodiversity conservation, the economic approach would suggest supporting the species that provide the highest quality service at the lowest cost. In Chapter 3 and Chapter 4 (Fig. S3.1, Table S4.1), wild pollinators in the genus *Lasioglossum* were the dominant pollinators and produced the largest strawberries. This implies that crop management efforts focused on increasing the abundance of *Lasioglossum* would provide the greatest yield increases in the short-term. Emerging research also indicates that relatively few regionally common species are responsible for providing the majority of crop pollination services, with rare species contributing very little (Kleijn et al. 2015; Winfree et al. 2015). This suggests that in many cases, economic-based ecosystem services arguments will be inadequate for wild pollinator conservation at large. Additionally, many studies do not consider the costs of maintaining wild pollinator populations on farms, or the relative costs and quality of service of managed versus wild pollinators (but see Isaacs et al. 2017), and yet economic factors are necessary to consider for the implementation of conservation initiatives. Further, several farm owners surveyed in Eastern Ontario (Chapter 5) had mutual rendering of service agreements, wherein a beekeeper provided pollination services in return for retaining honey production profits. Justifying the cost of enhancing wild pollinator services would be difficult in these situations.

6.3 Wild and managed bees in strawberry agroecosystems

Despite the agreements between strawberry farmers in Eastern Ontario, there are many regions and crops in which farmers pay to rent the services of managed honey bees, the cost of which has been steadily increasing (Melathopoulos et al. 2015). In these situations, wild bees may be a viable alternative or complement to managed crop pollination, provided their populations are sufficiently abundant in the farmed landscape. Conversely, overstocking honey bees in crops containing an abundance of efficient wild pollinators can have a negative effect on pollination quality and crop yields. For strawberry, I found that flowers visited by a honey bee first produced strawberries that were smaller than those visited by a wild bee first (Fig 3.5). This suggests that honey bees can usurp ovules that could have been fertilized by higher-quality (outcrossed) pollen had a wild bee visited the flower first. Thus, overstocking honey bees in systems with an abundance of wild pollinators may actually reduce crop yields. This finding adds to the limited literature on potential negative impacts of honey bee pollination on crop yields, relative to wild bee species (MacKenzie 1994; Saez et al. 2014).

The two investigations of wild versus managed bees in my research indicate that small wild bee species (primarily in the genus *Lasioglossum)* are effective, yield-increasing pollinators of strawberry. The mechanism driving this effect was related to specific foraging behaviours exhibited by these wild crop pollinators. *Lasioglossum* bees foraged between rows more often than honey bees; honey bees tended to forage in a straight line among strawberry plants in the same row (Chapter 4). This straight-line foraging pattern can reduce cross-pollination, and thus strawberry size, when one clonal variety of low self-compatibility is planted in succession (Fig

4.3). However, it was not possible to discern whether this type of foraging behaviour is characteristic of *Lasioglossum* bees, or whether competition with honey bees influenced their movements. Honey bees were abundant at the study site and it was difficult to accurately track all of the movements of the minuscule *Lasioglossum* bees. Planting multiple strawberry varieties in close proximity may positively influence cross-pollination rates and fruit yield whenever wild bees are present, but this possibility would need to be tested in the presence and absence of honey bees. Logistically, incorporating multiple varieties within or between rows would simply be a matter of organizing the varieties with the plant handlers, as commercial strawberry fields are usually planted by hand with the assistance of a mechanical transplanter. However, the potential of pollination to increase yield depends on the degree of self-compatibility within the variety (Figure 4.2). As there are approximately 250 commercial strawberries varieties cultivated in North America and over 600 varieties worldwide (StrawberryPlants.org, 2019), further testing would be required to determine the varietal crosses most conducive to increases in strawberry yield.

Irrespective of any interactive effects of field design and bee foraging behaviours, a precondition for strawberry growers to benefit from wild bee pollination is the sufficient abundance of wild bees. Landscapes that are more heterogenous are more likely to contain multiple habitat and floral resource options for pollinators, and consequently more diverse pollinator assemblages (Tscharntke et al. 2012). However, croplands are often highly simplified (homogeneous) landscapes with limited floral and nesting resources for bees, so cross-habitat spillover of bees from natural areas to managed areas becomes an important source of wild pollination (Blitzer et al. 2012). In strawberry, spillover may be essential for wild pollination as the crop is heavily mulched, which limits ground-nesting bee habitat within the crop. Thus, wild strawberry pollination likely originates from bees living in areas adjacent to the crop, but the potential for wild bees to nest under straw mulches needs to be investigated further.

The most common types of habitat in strawberry field edges in my study area (Southern Quebec and Eastern Ontario) were forest patches and hedgerows. Although larger expanses of natural land are expected to harbour more abundant and species-rich bee assemblages (Steffan-Dewenter 2003; Kremen et al. 2004), I did not find a difference in the wild bee richness or abundance between strawberry fields surrounded by hedgerows and those surrounded by larger expanses of forest (Chapter 5; Table 5.1; Fig 5.3a). One potential reason the bee communities did not differ with field-margin type is that species responses to the landscape changes could have been more influenced by the historical conversion of the landscape, rather than current differences in conditions (Aguirre-Gutiérrez et al. 2015). The bee community composition may have already been drastically altered when the land was transformed for farming, and the only remaining species are those well-adapted to agricultural conditions. Secondly, habitat remnants like hedgerows can be important sources of habitat and floral resources for bees in intensified farm systems, but in complex landscapes, the influence of heterogeneity at regional scales may overshadow the influence of habitat elements at the field scale (Tscharntke et al., 2005, Motzke et al. 2016). The landscapes surrounding my study sites would be considered complex as they contained a mosaic of forested, urban, and agricultural lands, with many small habitat islands (Fig 5.1). The complexity of the landscape at the regional scale may explain why I did not find a difference in bee communities between field-margin types at the field scale.

Bee responses to landscape composition are also scale- and body size-dependent (Steffan-Dewenter et al. 2002; Benjamin et al. 2014). Large-bodied bees (> 2.0 mm) respond negatively to increases in agricultural land at both the field and regional scales (Benjamin et al. 2104). In my study of the strawberry farmland (Chapter 5), I found fewer large-bodied bees at hedgerow sites than forested sites. As hedgerow sites contained more agricultural lands, this may explain why there were fewer large-bodied bees at these sites. However, the amount of natural habitat at the field scale was correlated to the amount of land at larger scales (1.2km radii) so the influence of each cannot be separated in my study. Small-bodied bees (ITD < 2.0 mm) are expected to be more affected by the composition of the land at the field scale due to their limited foraging ranges. Small-bodied wild bees were much less abundant than large-bodied bees or honey bees in field interiors, regardless of the type of habitat at the field edge. The number of honey bees was almost double that of all wild bees, thus the former were most likely providing more pollination than the latter at the field scale. The large field sizes (>200 m long), coupled with the limited foraging range of these small bees, limits their abundance in crop interiors, and their potential as strawberry pollinators.

To increase the abundance of small wild bees on strawberry farms in Eastern Canada, suitable habitat and floral resources would need to be provided in close proximity to the crop. Since *Lasioglossum* bees are ground-nesting, ideally habitat would be provided within the crop given typical field sizes in the study region. Incorporating ground-nesting bee habitat within the crop interior may be impossible given that conventional strawberry cultivation requires the crop to be covered with straw. However, strips of bare ground along crop margins may increase habitat options for these bees in smaller fields. Strawberry also blooms early in our region (mid-May), when non-crop floral resources are scarce (table 5.1). Efforts to increase the diversity and abundance of early-flowering plants (especially prior to crop bloom) may be rewarded with an increase in wild pollination. However, some field margin types can lead to increased pest and weed species that can spread into cropland (Marshall and Moonen 2002), and concurrently blooming plants may increase competition for pollination with the focal crop (Zhang et al. 2007). These possibilities were not investigated here, but I did find that pollen from the flowers blooming at crop margins did not transfer into the focal crop (Table 5.2), which diminishes concern surrounding heterospecific pollen transfer from non-crop plants at the field perimeter.

6.3 Conclusions and future research

The primary objective of my thesis was to explore how the composition of the bee community affects pollination and crop production, with a focus on strawberry agroecosystems. In Chapter 1, I provide a review of the literature on biodiversity and ecosystem services, particularly where it pertains to crop pollination. In Chapter 2, I developed a new pollen deposition measurement technique to facilitate my detailed investigation of pollen deposition by assemblages of bees. In Chapter 3, I conducted the first study to assess the influence of species richness and wild and managed bees on strawberry pollination using the pollen contribution of each bee in a sequence of visits. In Chapter 4, I addressed questions about the interaction between bee foraging behaviour and field design that had not previously been explored in cropping systems. In Chapter 5, I studied pollen depsotion at a larger scale than the previous chapters and compared the influence of common elements of the agricultural landscape (forests, hedgerows) on bee community composition. Although there are several studies of the effects of introducing field-edge habitat on pollinators, comparison studies between potential habitat types are rare.

As a large portion of my research constitutes a detailed investigation of pollination at the flower level, the ability to determine the impact of wild pollinators on strawberry pollination at the field scale is limited. Further, the data were collected over one growing season per chapter, so my findings surrounding species richness and pollination are only reliably applicable to the varieties and the wild bee community at my particular study sites and seasons. This work cannot address whether diversity within bee communities is a viable form of pollination insurance in the face of global change (e.g. Winfree et al. 2007). Additional research is necessary to investigate the general role of bee species richness on crop pollination in the long term, and multi-year studies are required to provide insight on the influences of species turnover and variability in bee community composition on the stability of ecosystem functions and services under conditions of environmental change.

Ecosystem services are generated from complex interactions between multiple providers across systems, and determining the interaction between service providers and their effects on multiple ecosystem services is beyond the scope of this study (see Nelson et al. 2015). However, understanding the relationships between specific ecosystem functions and subsequent services is necessary to manage ecosystems effectively for the well-being of humans and of multiple service providers. In the case of crop pollination, we need to understand the capacity of individual species and pollinator communities to provide pollination services at multiple scales. Detailed examinations of the interactions between pollinators and plants at the micro scale are essential to understanding the dynamics between pollinators and plants at broader scales.

My investigation of bee diversity and crop pollination highlights the complexities and challenges inherent in the study of pollination in the context of biodiversity and ecosystem services. The insights gained here, specifically about the influence of visit order on pollen deposition, and wild and managed bee foraging behaviour, can be incorporated into research at larger scales. The pollen measurement techniques and experiments designed here can be readily replicated across systems and timescales. It is hoped that the research compiled in this thesis will be used to improve accuracy in measuring pollination services and further knowledge on the relationship between pollinators and fruit production. With the ceaseless expansion of pollinator-dependent crops worldwide (Aizen et al. 2019), the management of agricultural landscapes for the conservation of effective crop pollinators is an important challenge for future research and for sustainable food production.

References

Agriculture and Agrifood Canada (AAFC). 2017. Crop profile for strawberry in Canada. Accessed June 2019. http://publications.gc.ca/collections/collection_2019/aac-aafc/A118-10-17-2016-eng.pdf

Aguirre-Gutiérrez, J., Biesmeijer, J.C., van Loon, E.E., Reemer, M., WallisDeVries, M.F., Carvalheiro, L.G., 2015. Susceptibility of pollinators to ongoing landscape changes depends on landscape history. Divers. Distrib. 21(10), 1129–1140.

Aizen, M.A., Harder, L.D., 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology 88(2), 271–281.

Aizen, M. A., Aguiar, S., Biesmeijer, J. C., Garibaldi, L. A., Inouye, D. W., Jung, C., [...], Pauw, A. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without an increase in crop diversification. Global Change Biology. Early View.

Aizen, M.A., Harder, L.D., 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19(11), 915–918.

Balvanera, P., Kremen, C., Martínez-Ramos, M., 2005. Applying community structure analysis to ecosystem function: examples from pollination and carbon storage. Ecol. Appl. 15(1), 360–375.

Barrett, S.C.H., Harder, L.D., 1996. Ecology and evolution of plant mating. Trends Ecol. Evol. 11(2), 73–79.

Bartomeus, I., Potts, S.G., Steffan-Dewenter, I., Vaissière, B.E., Woyciechowski, M., Krewenka, K.M., […], Bommarco, R., 2014. Contribution of insect pollinators to crop yield and quality varies with agricultural intensification. PeerJ 2, e328.

Bartomeus, I., Winfree, R., 2011. The Circe principle: are pollinators waylaid by attractive habitats? Curr. Biol. 21(17), R652–R654.

Benjamin, F., Reilly, J.R., Winfree, R., 2014. Pollinator body size mediates the scale at which land use drives crop pollination services. J. Appl. Ecol. 51(2), 440-449.

Blitzer, E. J., Dormann, C. F., Holzschuh, A., Klein, A. M., Rand, T. A., Tscharntke, T., 2012. Spillover of functionally important organisms between managed and natural habitats. Agr., Ecosyst. Environ. 146(1), 34-43.

Blüthgen, N., Klein, A-M., 2011. Functional complementarity and specialisation: The role of biodiversity in plant–pollinator interactions. Basic Appl. Ecol. 12(4), 282–291.

Bringhurst, R.S., Ahmadi, H., Voth, V., 1988. Inheritance of the day-neutral trait in strawberries. Acta Hortic. 265, 35–42.

Brittain, C., Williams, N., Kremen, C., Klein, A.-M., 2013. Synergistic effects of non-Apis bees and honey bees for pollination services. Proc. R. Soc. Lond., B, Biol. Sci. 280(1754), 20122767

Burd, M., 1994. Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. Bot. Rev. 60(1), 83–139.

Burd, M., 2008. The Haig-Westoby model revisited. Am. Nat. 171(3), 400–4.

Cardinale, B.J., Srivastava, D.S., Emmett Duffy, J., Wright, J.P., Downing, A.L., Sankaran, M., Jouseau, C., 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 443(7114), 989–992.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., [...], Kinzig, A. P., 2012. Biodiversity loss and its impact on humanity. Nature, 486(7401), 59-67.

Carvalheiro, L.G., Veldtman, R., Shenkute, A.G., Tesfay, G.B., Pirk, C.W.W., Donaldson, J.S., Nicolson, S.W., 2011. Natural and within-farmland biodiversity enhances crop productivity. Ecol. Lett. 14(3), 251–259.

Chacoff, N.P., Aizen, M.A., 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. J. Appl. Ecol. 43(1), 18–27.

Chagnon, M., Gingras, J., De Oliveira, D., 1989. Effect of honey bee (hymenoptera: apidae) visits on the pollination rate of strawberries. J. Econ. Entomol. 82(5), 1350–1353.

Chagnon, M., Gingras, J., DeOliveira, D., 1993. Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). J. Econ. Entomol. 86(2), 416–420.

Clarke, D.A., York, P.H., Rasheed, M.A., Northfield, T.D., 2017. Does biodiversity–ecosystem function literature neglect tropical ecosystems? Trends Ecol. Evol. 32(5), 320–323.

Colbert, S., de Oliveira, D. 1992. Pollinisation croisée et production de quatre cultivars de fraisier, *Fragaria* × *ananassa*. Can. J. Plant Sci. 72(3), 857-861.

Cusser, S., Neff, J. L., Jha, S., 2016. Natural land cover drives pollinator abundance and richness, leading to reductions in pollen limitation in cotton agroecosystems. Agr., Ecosyst. Environ. 226, 33-42.

Darrow, G.M., 1966. *The Strawberry*. Holt, Rinehart and Winston, New York, NY.

Darwin, C.R. 1876. *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. John Murray, London.

David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E.L., Hill, E.M., Goulson, D., 2016. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. Environ. Int. 88, 169–178.

de Groot, R.S., Wilson, M.A., Boumans, R.M.., 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecol. Econ. 41(3), 393–408.

Eilers, E.J., Kremen, C., Smith Greenleaf, S., Garber, A.K., Klein, A.-M., 2011. Contribution of pollinator-mediated crops to nutrients in the human food supply. PLoS One 6(6), e21363.

Elton, C. S. 1958. The ecology of invasions by animals and plants. University of Chicago Press, Chicago, IL.

Fahrig, L., Girard, J., Duro, D., Pasher, J., Smith, A., Javorek, S., King, D., Lindsay, K.F., Mitchell, S., Tischendorf, L., 2015. Farmlands with smaller crop fields have higher within-field biodiversity. Agric. Ecosyst. Environ. 200, 219–234.

Fisher, B., Kerry Turner, R., 2008. Ecosystem services: classification for valuation. Biol. Conserv. 141(5), 1167–1169.

Fontaine, C., Dajoz, I., Meriguet, J., Loreau, M., 2005. Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. PLoS Biol. 4(1), e1.

Forrest, J.R.K., Thorp, R.W., Kremen, C., Williams, N.M., 2015. Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape. J. Appl. Ecol. 52(3), 706–715.

Free, J.B., 1968. The pollination of strawberries by honey-bees. J. Hortic. Sci. 43(1), 107–111.

Fründ, J., Dormann, C.F., Holzschuh, A., Tscharntke, T., 2013. Bee diversity effects on pollination depend on functional complementarity and niche shifts. Ecology 94(9), 2042–2054.

Garibaldi, L.A., Aizen, M.A., Klein, A.M., Cunningham, S.A., Harder, L.D., 2011. Global growth and stability of agricultural yield decrease with pollinator dependence. Proc. Natl. Acad. Sci. U.S.A. 108(14), 5909–5914.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., […], Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127), 1608–1611.

Garibaldi, L.A., Carvalheiro, L.G., Vaissière, B.E., Gemmill-Herren, B., Hipólito, J., Freitas, B.M., […], Zhang, H., 2016. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. Science 351, 388–391.

Gathmann, A., Tscharntke, T., 2002. Foraging ranges of solitary bees. J. Anim. Ecol. 71(5), 757–764.

Goodman, R., Oldroyd, B., 1988. Honeybee pollination of strawberries (Fragaria x ananassa Duchesne). Aust. J. Exp. Agric. 28(3), 435-438.

Greenleaf, S.S., Kremen, C., 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. U.S.A. 103(37), 13890–13895.

Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia, 153(3), 589–596.

Grime, J. P. 1997. Biodiversity and ecosystem function: the debate deepens. Science 277(5330), 1260-1261.

Haig, D., Westoby, M., 1988. On limits to seed production. Am. Nat. 131(5), 757–759.

Hendrickx, F., Maelfait, J.-P., Van Wingerden, W., Schweiger, O., Speelmans, M., Aviron, S., […], Bugter, R., 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. J. Appl. Ecol. 44(2), 340–351.

Hoehn, P., Tscharntke, T., Tylianakis, J. M., & Steffan-Dewenter, I. 2008. Functional group diversity of bee pollinators increases crop yield. Proc. R. Soc. Lond., B, Biol. Sci. 275(1648), 2283-2291.

Holzschuh, A., Steffan-Dewenter, I., Tscharntke, T., 2008. Agricultural landscapes with organic crops support higher pollinator diversity. Oikos 117(3), 354-361.

Hooper, D.U., Vitousek, P.M., 1997. The effects of plant composition and diversity on ecosystem processes. Science 277(5330), 1302–1305.

Horth, L., Campbell, L.A., 2018. Supplementing small farms with native mason bees increases strawberry size and growth rate. J. Appl. Ecol. 55(2), 591–599.

Huston, M.A., 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110(4), 449–460.

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2016. Assessment Report on Pollinators, Pollination and Food Production. Simon G. Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T. (eds.). IPBES secretariat, Bonn, Germany

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2019. Global assessment report on biodiversity and ecosystem services. S. Díaz, J. Settele, E. S. Brondizio E.S., H. T. Ngo, M. Guèze, J. Agard, A. Arneth, […], Zayas, C.N. (eds.). IPBES secretariat, Bonn, Germany

Isaacs, R., Williams, N., Ellis, J., Pitts-Singer, T. L., Bommarco, R., Vaughan, M., 2017. Integrated crop pollination: combining strategies to ensure stable and sustainable yields of pollination-dependent crops. Basic Appl. Ecol. 22, 44-60.

Ives, A.R., Cardinale, B.J., Snyder, W.E., 2004. A synthesis of subdisciplines: predator-prey interactions, and biodiversity and ecosystem functioning. Ecol. Lett. 8(1), 102–116.

Jones, C. G., Lawton, J. H., Shachak, M. 1994. Organisms as ecosystem engineers. In *Ecosystem management*, 130-147. Springer, New York, NY.

Kennedy, C.M., Lonsdorf, E., Neel, M.C., Williams, N.M., Ricketts, T.H., Winfree, […], Kremen, C., 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol. Lett. 16(5), 584–599.

King, C., Ballantyne, G., Willmer, P.G., 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. Methods Ecol. Evol. 4(9), 811–818.

Klatt, B.K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., Tscharntke, T., 2013. Bee pollination improves crop quality, shelf life and commercial value. Proc. R. Soc. Lond., B, Biol. Sci. 281(1775), 20132440.

Kleijn, D., Baquero, R.A., Clough, Y., Díaz, M., Esteban, J., Fernández, F., […], Yela, J.L., 2006. Mixed biodiversity benefits of agri-environment schemes in five European countries. Ecol. Lett. 9(3), 243–254.

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.-M., […], Potts, S.G., 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 7414.

Klein, A.M., Brittain, C., Hendrix, S.D., Thorp, R., Williams, N., Kremen, C., 2012. Wild pollination services to California almond rely on semi-natural habitat. J. Appl. Ecol. 49(3), 723–732.

Klein, A.M., Steffan-Dewenter, I., Tscharntke, T., 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proc. R. Soc. Lond., B, Biol. Sci. 270(1518), 955–961.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2006. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond., B, Biol. Sci. 274(1608), 303-313.

Kohler, F., Verhulst, J., Klink, R. Van, Kleijn, D., 2008. At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes? J. Appl. Ecol. 45(3), 753–762.

Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P., Thorp, R. W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecology letters, 7(11), 1109-1119.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., […], Ricketts, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol. Lett. 10(4), 299–314.

Lander, T.A., Bebber, D.P., Choy, C.T.L., Harris, S.A., Boshier, D.H., 2011. The Circe principle explains how resource-rich land can waylay pollinators in fragmented landscapes. Curr. Biol. 21(5), 1302–1307.

Landis, D.A., 2017. Designing agricultural landscapes for biodiversity-based ecosystem services. Basic Appl. Ecol. 18, 1–12.

Lanz, B., Dietz, S., & Swanson, T. 2018. The expansion of modern agriculture and global biodiversity decline: An integrated assessment. Ecol. Econ. 144, 260-277.

Levin, D.A., Kerster, H.W., Niedzlek, M., 1971. Pollinator flight directionality and its effect on pollen flow. Evolution 25(1), 113-118.

Levin, D. A., 1979. Pollinator foraging behavior: genetic implications for plants. In *Topics in Plant Population Biology*, 131-153. Palgrave, London, UK.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., […], Wardle, D.A., 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294(5543), 804–808.

Mace, G.M., Norris, K., Fitter, A.H., 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends Ecol. Evol. 27(1), 19–26.

MacFadyen, S., Cunningham, S.A., Costamagna, A.C., Schellhorn, N.A., 2012. Managing ecosystem services and biodiversity conservation in agricultural landscapes: Are the solutions the same? J. Appl. Ecol. 49(3), 690–694.

MacKenzie, K.E., 1994. The foraging behaviour of honey bees (*Apis mellifera L)* and bumble bees *(Bombus spp)* on cranberry *(Vaccinium macrocarpon* Ait*)*. Apidologie 25(4), 375-383.

Mallinger, R.E. & Gratton, C., 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. J. Appl. Ecol. 52(2), 323–330.

Marshall, E.J.P., Moonen, A.C., 2002. Field margins in northern Europe: their functions and interactions with agriculture. Agr., Ecosyst. Environ. 89(1-2), 5-21.

Martin, E.A., Dainese, M., Clough, Y., Báldi, A., Bommarco, R., Gagic, V., […], Steffan-Dewenter, I., 2019. The interplay of landscape composition and configuration: new pathways to manage functional biodiversity and agroecosystem services across Europe. Ecol. Lett. 22(7), 1083-1094.

Martins, K. T., Gonzalez, A., & Lechowicz, M. J., 2015. Pollination services are mediated by bee functional diversity and landscape context. Agr., Ecosyst. Environ. 200, 12-20

McKinney, M.L., 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. Annu. Rev. Ecol. Syst. 28(1), 495–516.

Melathopoulos, A. P., Cutler, G. C., & Tyedmers, P., 2015. Where is the value in valuing pollination ecosystem services to agriculture? Ecol. Econ. 109, 59-70.

Millennium Ecosystem Assessment, 2005. Ecosystems and human well-being. Washington, DC: Island Press.

Morandin, L.A., Kremen, C., 2013. Hedgerow restoration promotes pollinator populations and exports native bees to adjacent fields. Ecol. Appl. 23(4), 829–839.

Morandin, L.A., Winston, M.L., 2006. Pollinators provide economic incentive to preserve natural land in agroecosystems. Agric. Ecosyst. Environ. 116(3-4), 289–292.

Naeem, S., 2002. Ecosystem consequences of biodiversity loss: the evolution of a paradigm. Ecology 83(6), 1537–1552.

Ne’eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S.G., Dafni, A., 2010. A framework for comparing pollinator performance: effectiveness and efficiency. Biol. Rev. 85(3), 435-451.

Nelson, E., Mendoza, G., Regetz, J., Polasky, S., Tallis, H., Cameron, D., [...], Lonsdorf, E., 2009. Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. Front. Ecol. Environ. 7(1), 4-11.

Nicholls, C.I., Altieri, M.A., 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agron. Sustain. Dev. 33(2), 257–274.

Nitsch, J.P., 1950. Growth and morphogenesis of the strawberry as related to auxin. Am. J. Bot. 37(3), 211–215.

Ontario ministry of Agriculture, Food and Rural Affaris (OMAFRA). 2016. Dayneutral Strawberries. Production Factsheet. Accessed June 2019: http://www.omafra.gov.on.ca/english/crops/facts/89-099.htm

Pasquet, R.S., Peltier, A., Hufford, M.B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J.T., Herren, H.R., Gepts, P., 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. Proc. Natl. Acad. Sci. 105(36), 13456–13461.

Pilling, E.D., Jepson, P.C., 1993. Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis mellifera* L.). Pestic. Sci. 39(4), 293–297.

Pion, S., Oliveira, D.D., Paradis, R. O., 1980. Agents pollinisateurs et productivité du fraisier 'Redcoat', *Fragaria* x *ananassa* Duch. Phytoprotection 61, 72–78.

Raw, A., 2000. Foraging Behaviour of Wild Bees at Hot Pepper Flowers (*Capsicum annuum*) and its Possible Influence on Cross Pollination. Ann. Bot. 85(4), 487–492.

Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., […], A., Viana, B.F., 2008. Landscape effects on crop pollination services: are there general patterns? Ecol. Lett. 11(5), 499–515.

Rogers, S.R., Tarpy, D.R., Burrack, H.J., 2014. Bee species diversity enhances productivity and stability in a perennial Crop. PLoS One 9(5), e97307.

Rundlöf, M., Nilsson, H., Smith, H.G., 2008. Interacting effects of farming practice and landscape context on bumble bees. Biol. Conserv. 141(2), 417–426.

Sáez, A., Morales, C. L., Ramos, L. Y., Aizen, M. A., 2014. Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. J. Appl. Ecol. 51(6), 1603-1612.

Scheper, J., Holzschuh, A., Kuussaari, M., Potts, S.G., Rundlöf, M., Smith, H.G., Kleijn, D., 2013. Environmental factors driving the effectiveness of European agri-environmental measures in mitigating pollinator loss - a meta-analysis. Ecol. Lett. 16(7), 912–920.

Schmitt, J., 1980. Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae). Evolution 34(5), 934–943.

Schulze, E. D., Mooney, H.A. 1993. *Biodiversity and ecosystem function*. Springer-Verlag. New York, NY.

Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M.T., Tosi, S., Bogo, G., […], Bosch, J., 2017. Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Manag. Sci. 73(6), 1236–1243.

Song, X.-P., Hansen, M.C., Stehman, S. V., Potapov, P. V., Tyukavina, A., Vermote, E.F., Townshend, J.R., 2018. Global land change from 1982 to 2016. Nature 560(7720), 639–643.

Srivastava, D.S., Vellend, M., 2005. Biodiversity-ecosystem function research: is it relevant to conservation? Annu. Rev. Ecol. Evol. Syst. 36, 267–294.

Steffan-Dewenter, I., Munzenberg, U., Burger, C., Thies, C., Tscharntke, T., 2002. Scale-dependent effects of landscape context on three pollinator guilds. Ecology, 83(5), 1421–1432.

Steffan-Dewenter, I., 2003. Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. Conserv. Biol. 17(4), 1036-1044.

StrawberryPlants.org, 2019. Strawberry Varieties. Retrieved June 2019, URL: https://strawberryplants.org/strawberry-varieties/

Sutter, L., Albrecht, M., 2016. Synergistic interactions of ecosystem services: florivorous pest control boosts crop yield increase through insect pollination. Proc. R. Soc. Lond., B, Biol. Sci. 283(1824), 20152529.

Swift, M.J., Izac, A.-M.N., van Noordwijk, M., 2004. Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions? Agric. Ecosyst. Environ. 104(1), 113–134.

Thomson, J.D., 1986. Pollen transport and deposition by bumble bees in erythronium: influences of floral nectar and bee grooming. J. Ecol. 74, 329-341.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277(5330), 1300-

Tilman, D., Downing, J.A., 1994. Biodiversity and stability in grasslands. Nature 367(6461), 363–365.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379(6567), 718–720.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity-ecosystem service management. Ecol. Lett. 8(8), 857–874.

Tscharntke, T., Tylianakis, J. M., Rand, T. A., Didham, R. K., Fahrig, L., Batary, P., [...], Ewers, R. M., 2012. Landscape moderation of biodiversity patterns and processes-eight hypotheses. Biol. Rev. 87(3), 661-685.

United States Department of Agriculture (USDA). 2017. Pesticide Data Program. Accessed June 2019.

Vandermeer, J., van Noordwijk, M., Anderson, J., Ong, C., Perfecto, I., 1998. Global change and multi-species agroecosystems: Concepts and issues. Agric. Ecosyst. Environ. 67(1), 1–22.

Vincent, C., Oliveira, D.D. de, Bélanger, A., 1990. The management of insect pollinators and pests in Quebec strawberry plantations. *In Monitoring and integrated management of arthropod pests of small fruit crops*, 177–192. Intercept Ltd. Andover, UK.

Visscher, P. K., & Seeley, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology, 63(6), 1790-1801.

Wardle, D.A., 2016. Do experiments exploring plant diversity-ecosystem functioning relationships inform how biodiversity loss impacts natural ecosystems? J. Veg. Sci. 27(3), 646–653.

Waser, N.M., Price, M. V., 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (polemoniaceae): are ovules usurped? Am. J. Bot. 78(8), 1036–1043.

Westphal, C., Steffan-Dewenter, I., Tscharntke, T., 2006. Foraging trip duration of bumblebees in relation to landscape-wide resource availability. Ecol. Entomol. 31(4), 389–394.

Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. Trends Plant Sci. 7(6), 270–277.

Winfree, R., Williams, N. M., Dushoff, J., & Kremen, C., 2007. Native bees provide insurance against ongoing honey bee losses. Ecol. Lett. 10(11), 1105-1113.

Winfree, R., Kremen, C., 2008. Are ecosystem services stabilized by differences among species? A test using crop pollination. Proc. R. Soc. Lond., B, Biol. Sci. 276(1655), 229-237.

Winfree, R., W. Fox, J., Williams, N. M., Reilly, J. R., & Cariveau, D. P., 2015. Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. Ecology letters, 18(7), 626-635.

Wolfe, L.M., Barrett, S.C.H., 1989. Patterns of pollen removal and deposition in tristylous *Pontederia cordata* L. (Pontederiaceae). Biol. J. Linn. Soc. 36(4), 317–329.

Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E., Desneux, N., 2012. Pollinator habitat enhancement: Benefits to other ecosystem services. Agric. Ecosyst. Environ. 159, 112–122.

Zebrowska, J., 1998. Influence of pollination modes on yield components in strawberry (*Fragaria* x *ananassa* Duch.). Plant Breed. 117(3), 255–260.

Zhang, W., Ricketts, T.H., Kremen, C., Carney, K. and Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. Ecol. Econ. 64(2), 253-260.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., Dorn, S., 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Biol. Conserv. 143(3), 669–676.

Appendix

Supplementary information for Chapter 3: Pollination by wild bees yields larger strawberries than pollination by honey bees

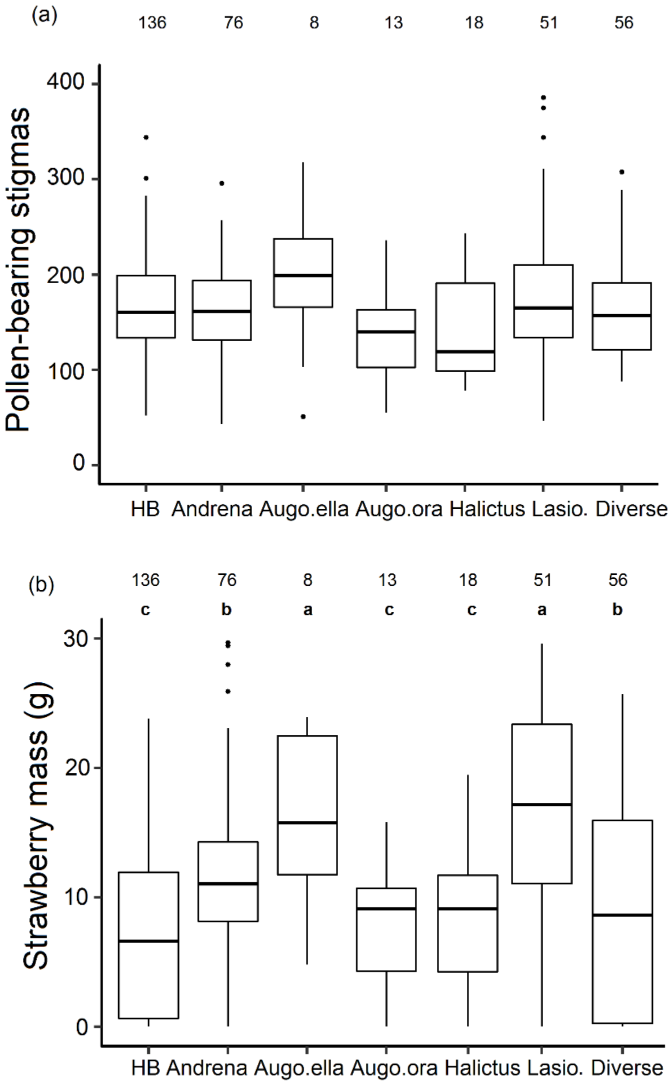

Figure S3.1. Pollen deposition (a) and strawberry mass (b) for flowers visited by different bee genera. Flowers and strawberries were categorized by the genus that comprised the majority (>50%) of visits received by the flower: Honey bees (HB), *Andrena*, *Augochlora* (Augo.ora), *Augochlorella* (Augo.ella), *Halictus*, or *Lasioglossum* (Lasio). "Diverse" flowers did not have a single majority visitor. Boxes show the interquartile range, the median is indicated by a horizontal line, whiskers indicate the data range, and points are outliers. Sample sizes are listed above. Letters indicate significant differences - genera with the same letter do not differ significantly in strawberry mass. There were no significant differences in pollen deposition by genera.

Table S3.1. Species list with family and abundance for bee species observed in this study in order of decreasing abundance. N is the number of individual flower visits by each species.

Species	**Family**	**N**
Apis mellifera Linnaeus	Apidae	362
Lasioglossum viridatum (species group)	Halictidae	43
Andrena imitatrix Cresson	Andrenidae	41
Andrena carlini Cockerell	Andrenidae	22
Andrena vicina Smith	Andrenidae	20
Augochlorella aurata (Smith)	Halictidae	18
Andrena nasonii Robertson	Andrenidae	15
Halictus confuses Smith	Halictidae	13
Augochlora pura (Say)	Halictidae	13
Hylaeus annulatus (Linnaeus)	Colletidae	11
Andrena persimulata Viereck	Andrenidae	10
Andrena brevipalpis Cockerell	Andrenidae	9
Andrena regularis Malloch	Andrenidae	8
Lasioglossum cattellae (Ellis)	Halictidae	8
Andrena commoda Smith	Andrenidae	7
Andrena cressoni cressonii Robertson	Andrenidae	7
Lasioglossum macoupinense (Robertson)	Halictidae	7
Andrena mandibularis Robertson	Andrenidae	6
Andrena robertsonii Dalla Torre	Andrenidae	6
Lasioglossum tegulare (Robertson)	Halictidae	6
Lasioglossum pectinatum (Robertson)	Halictidae	5
Andrena fragilis Smith	Andrenidae	4
Halictus ligatus Say	Halictidae	4
Lasioglossum georgeickworti Gibbs	Halictidae	3
Andrena integra Smith	Andrenidae	2

Andrena platyparia Robertson	Andrenidae	2
Halictus rubicundus Christ	Halictidae	2
Lasioglossum pectorale (Smith)	Halictidae	2
Lasioglossum sheffieldi Gibbs	Halictidae	2
Lasioglossum albipenne (Robertson)	Halictidae	1
Andrena melanochroa Cockerell	Andrenidae	1
Andrena ziziae Robertson	Andrenidae	1
Bombus impatiens Cresson	Apidae	1
Bombus rufocinctus Cresson	Apidae	1

Table S3.2. Mean and standard deviation of total pollen-bearing strawberry stigmas and strawberry mass as functions of visitor species richness (Chapter 3). Neither the total number of pollen-bearing stigmas nor strawberry mass changed with species richness of flower visitors. The reference group was 1 visitor species.

No. of species	**Pollen-bearing stigmas (mean ± SD)**	**n**	**z (p)**	**Strawberry mass (g) (mean ± SD)**	**n**	**z (p)**
1	160.80 ± 64.22	10		14.81 ± 8.43	10	
2	208.80 ± 70.46	31	0.38 (0.85)	14.92 ± 9.02	31	1.97 (0.09)
3	162.40 ± 59.41	30	0.29 (0.91)	11.63 ± 6.63	30	1.61 (0.18)
4	166.52 ± 60.84	27	0.16 (0.98)	8.77 ± 7.14	27	1.13 (0.40)

Table S3.3. Mean and standard deviation of net and total pollen-bearing strawberry stigmas for honey bee and wild bee groups per flower, per visit (Chapter 3). The amount of autogamous (self) pollen within each flower prior to sampling is also listed. n is the number of visits per visit level. The total pollen measurements for the control groups are included below.

Visit	Type	Net pollen (mean ± SD)	n	Total pollen (mean ± SD)	n	Self pollen (mean ± SD)	n
1	HB	28.28 ± 24.64	81	126.17 ± 54.72	23	91.78 ± 66.79	23
	WB	25.35 ± 21.47	54	122.49 ± 42.35	34	94.12 ± 44.14	34
2	HB	25.15 ± 25.91	61	161.83 ± 44.94	29	100.07 ± 48.98	29
	WB	19.39 ± 16.84	44	177.06 ± 65.38	17	130.00 ± 78.69	17
3	HB	19.19 ± 22.10	32	171.62 ± 60.55	21	114.71 ± 63.98	21
	WB	18.21 ± 18.13	34	148.29 ± 49.58	17	91.94 ± 40.91	17
4	HB	12.50 ± 12.11	22	175.12 ± 29.22	16	100.19 ± 33.68	16
	WB	7.30 ± 10.79	20	185.67 ± 46.91	10	97.70 ± 33.55	10
6	HB	3.79 ± 5.79	14	175.08 ± 40.88	26	96.62 ± 44.55	26
	WB	6.52 ± 5.59	17	171.95 ± 51.12	19	98.95 ± 45.92	19
Controls	Hand	NA	NA	193.53 ± 40.54	22	NA	NA
	Open	NA	NA	185.25 ± 62.89	24	NA	NA
	Bagged	NA	NA	106.00 ± 46.98	20	NA	NA

Table S3.4. Mean and standard deviation of strawberry mass as a function of number of bee visits for wild bees (WB) and honey bees (HB). The results of the GLM comparisons between bee types (WB as the reference group) and the total visits received per flower are listed: test statistic (z), sample size (n) and p-value (p).

Visit no.	**WB (mean ± SD)**	**n**	**HB (mean ± SD)**	**n**	**z**	**p**
1	14.45 ± 7.78	34	7.42 ± 7.80	23	2.87	<0.001
2	13.64 ± 11.19	17	8.12 ± 6.64	29	2.26	0.029
3	11.33 ± 8.37	17	8.63 ± 5.37	21	2.12	0.041
4	13.06 ± 8.50	9	7.41 ± 7.74	16	1.79	0.087
6	10.25 ± 5.01	20	5.92 ± 4.05	26	4.43	<0.001

Supplementary information for Chapter 5: Small wild bees decline with distance into strawberry crops regardless of field margin habitat

Table S5.2. Species list of plants in flower at strawberry fields with hedgerow and forested margins

	Flowering shrubs	**Trees**	**Herbaceous**
Hedgerow	*Cornus alternifolia* L.f. *Cornus sericea* L *Viburnum* spp. *Lonicera tatarica* L. *Rubus occidentalis* L.	*Malus domestica* Borkh *Crataegus mollis* (Torr. & Gray) Scheele	*Taraxacum officinale* (L.) Weber ex F.H. Wigg *Barbarea vulgaris* W. T. Aiton *Viola sororia* Willd. *Hesperis matronalis* L. *Alliaria petiolata* (M.Bieb.) Cavara & Grande *Thlaspi arvense* L. *Fragaria vesca* L.
Forest	*Lonicera tatarica* L. *Rubus occidentalis* L.	*Acer saccharum* Marshall *Populus tremuloides* Michx. *Betula papyrifera* Marshall *Fraxinus pennsylvanica* Marshall	*Taraxacum officinale* (L.) Weber ex F.H. Wigg *Barbarea vulgaris* W. T. Aiton *Viola sororia* Willd. *Hesperis matronalis* L. *Alliaria petiolata* (M.Bieb.) Cavara & Grande *Thlaspi arvense* L. *Fragaria vesca* L.

Table S5.1. Species list, with the number of female (F) and male (M) specimens observed (*Apis mellifera* L.) or collected (all others) and the average inter-tegular distance in millimeters (mm)

Genus	**Species**	**Individuals**		**ITD (mm)**
		(F)	**(M)**	
Apis	*mellifera* Linnaeus	614		3.6
Lasioglossum		269		
Lasioglossum	*versatum* (Robertson)	70		1.9
Lasioglossum	*tegulare* (Robertson)	44	1	1.0
Lasioglossum	*perpunctatum* (Ellis)	33		1.6
Lasioglossum	*hitchensi* Gibbs	20		1.6
Lasioglossum	*foxii* (Robertson)	16		1.6
Lasioglossum	*bruneri* (Crawford)	13		1.7
Lasioglossum	*imitatum* (Smith)	13		1.0
Lasioglossum	*leucozonium* (Schrank)	10		2.5
Lasioglossum	*versans* (Lovell)	9		1.6
Lasioglossum	*cressonii* (Robertson)	6		2.1
Lasioglossum	*pectorale* (Smith)	6		1.5
Lasioglossum	*zonulum* (Smith)	4		2.5
Lasioglossum	*coriaceum* (Smith)	3		2.4
Lasioglossum	*laevissimum* (Smith)	3		1.4
Lasioglossum	*timothyi* Gibbs	3		1.4
Lasioglossum	*dresbachi* (Mitchell)	2		1.0
Lasioglossum	*lineatulum* (Crawford)	2		1.5
Lasioglossum	*macoupinense* (Robertson)	2		1.7
Lasioglossum	*planatum* (Lovell)	2		1.0
Lasioglossum	*sagax* (Sandhouse)	2		1.6
Lasioglossum	*tenax* (Sandhouse)	2		1.0

Lasioglossum	*katherinae* Gibbs	1		1.0
Lasioglossum	*oblongum* (Lovell)	1		1.6
Lasioglossum	*viridatum* (Lovell)	1		1.6
Lasioglossum	*weemsi* (Mitchell)	1		1.5
Andrena		218		
Andrena	*nasonii* Robertson	78		1.65
Andrena	*cressonii* Robertson	38		2.72
Andrena	*carlini* Cockerell	24		3.7
Andrena	*commoda* Smith	16		4.0
Andrena	*hippotes* Robertson	13		2.8
Andrena	*mandibularis* Robertson	11		2.2
Andrena	*distans* Provancher	6		1.5
Andrena	*nivalis* Smith	6		2.75
Andrena	*forbesii* Robertson	4		3.0
Andrena	*regularis* Malloch	4		3.5
Andrena	*wheeleri* Graenicher	4		2.3
Andrena	*imitatrix* Cresson	3	1	1.6
Andrena	*perplexa* Smith	3		3.2
Andrena	*rufosignata* Cockerell	3		2.6
Andrena	*frigida* Smith	1		3.0
Andrena	*rugosa* Robertson	1		3.3
Andrena	*vicina* Smith	1		3.4
Andrena	*wilkella* (Kirby)	1		2.3
Andrena	*w-scripta* Viereck	1		2.7
Halictus		126		
Halictus	*confusus* Smith	74		1.9
Halictus	*rubicundus* Christ	39		3.1
Halictus	*ligatus* Say	13		2.4
Ceratina		62		

Ceratina	*calcarata* Robertson	40	2	1.9
Ceratina	*dupla* Say	22		1.4
Agapostemon		29		
Agapostemon	*sericeus* Forster	18		2.8
Agapostemon	*texanus* Cresson	6		2.7
Agapostemon	*virescens* (Fabricius)	4		3.4
Agapostemon	*splendens* (Lepeletier)	1		2.6
Augochlorella	*aurata* (Smith)	26		2.0
Augochlora	*pura* (Say)	2		2.5
Bombus		21		
Bombus	*rufocinctus* Cresson	17		7.4
Bombus	*impatiens* Cresson	4		8.6
Osmia		16		
Osmia	*atriventris* Cresson	6		2.7
Osmia	*laticeps* Thomson	3	1	2.4
Osmia	*pumila* Cresson	6		2.4
Osmia	*simillima* Smith	1		3.3
Hoplitis		3		
Hoplitis	*producta* (Cresson)	2		2.1
Hoplitis	*truncata* (Cresson)	1		2.1
Hylaeus	*annulatus* (Linnaeus)	3		1.2
Colletes	*inequalis* Say	3		3.7
Megachile	*melanophaea* Smith		1	4.4
Nomada		3		
Nomada	*integerrima* Dalla		1	1.5
Nomada	*maculata* Cresson	2	1	3.0
Sphecodes		2		
Sphecodes	*minor* Robertson	1	1	1.5
Sphecodes	*solonis* Graenicher	1		1.5

Table S5.3. Results of GLMs comparing the 20 most abundant bee species over distances and field margin types. Only the abundance of species significantly different between sites and distances are shown. ITD is the average inter-tegular distance for each species. Likelihood ratio tests (LRT) provided the test statistics and p is the significance value, in bold if < 0.05.

Species	**Forest (no. individuals)**	**Hedge (no. individuals)**	**LRT**	**p**
Agapostemon sericeus	17	2	7.98	**0.007**
Andrena carlini	18	6	4.82	**0.028**
Augochlorella aurata	22	3	9.58	**0.004**

	Distance						
	0 m	**50 m**	**100 m**	**150 m**	**ITD**		
Andrena cressonii	14	15	7	1	2.72	5.24	**0.019**
Andrena hippotes	8	5	0	0	2.80	5.77	**0.023**
Ceratina calcarata	30	6	4	0	1.90	6.11	**0.020**
Lasioglossum bruneri	12	0	1	0	1.70	8.28	**0.011**
Lasioglossum imitatum	10	0	2	1	1.00	6.82	**0.003**
Lasioglossum tegulare	25	7	10	2	1.00	2.192	0.097